THE
ETIOLOGY AND PATHOLOGY
OF TYPHUS

BEING THE MAIN REPORT OF THE
TYPHUS RESEARCH COMMISSION OF THE LEAGUE
OF RED CROSS SOCIETIES TO POLAND

BY

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Miss STELLA MATTHEWS, Chief Nurse; and the following Nursing Staff:
Nurses ALLEN, CALHOUN, CRAUFURD, JONES, MEEKE, RHODE,
RISDEN, ROE, ROLLINS, RUST, SUTTON, and TUTT
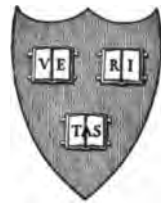
FOREWORD AND ACKNOWLEDGMENTS

The decision of the League of Red Cross Societies to send a commission to Poland for the purpose of making researches upon Typhus was initiated by Richard P. Strong then Medical Director of the League of Red Cross Societies.

The laboratory members of the Commission sailed from New York on the thirtieth of January, 1920 with the intention of returning in July or August. Owing to delays caused by difficulties of transportation and supply the Commission did not arrive in Warsaw until the twenty-fifth of February. The original destination was Vilna, but after a brief visit there it was decided that better opportunities for the work were to be had in Warsaw through the facilities offered by the Polish Government. There were still many difficulties to be overcome and not until the end of March were observations begun.

On every side, interest in and willingness to assist the work was evidenced. Thanks are due from the members of the Commission to Mr. Hugh Gibson, American Minister to Poland, Sir Horace Rumbold, British Minister to Poland, Mr. William C. Boyden, Commissioner of the League of Red Cross Societies to Poland, Colonel Henry A. Shaw, Chief of Sanitary Department of the League of Red Cross Societies in Poland, and to Colonel A. J. Chesley, American Red Cross Commissioner to Poland; it is especially wished to express thanks to the staff of the American Red Cross in Poland, who individually willingly met many calls for help.

Without the keen coöperation received from the Polish authorities, the work of the Commission would have been impossible. By every representative of the Polish Government, from the Chief of State, General Pilsudski, downwards, earnest and intelligent assistance was constantly evidenced. Excellent facilities for the Commission's work were provided in Warsaw. Two wards of the St. Stanislaus Typhus Hospital were placed entirely under its control. Very ample laboratory space was

provided in the new building of the Central State Epidemiological Institute. For these facilities, and for many other courtesies, we are indebted to Dr. Witold Chodzko, Minister of Health, to the Minister of Foreign Affairs, Mr. Patek, to the Sisters of St. Vincent de Paul and the Medical Staff of the St. Stanislaus Hospital, and to Dr. Ludwig Rajchman in whose Institute the laboratory work of the Commission was done. We are especially grateful to Dr. Amaiela Apatow of the St. Stanislaus Hospital Staff for many services and great personal interest in promoting the success of our work.

Finally, it is especially desired to acknowledge the sympathy with our work shown by the medical profession of Warsaw. We are grateful to them for the interest which they took in the preparations and results which were put before them from time to time as our study progressed.

It is also desired to acknowledge the promptness and completeness met by requests for assistance sent, during the work in Warsaw, to the headquarters of the League of Red Cross Societies in Geneva, or to the head office of the American Red Cross in Paris. The latter acted as our base for supplies.

After leaving Warsaw, demonstrations of the Commission's work were given at the Pasteur Institute of Paris, and at the Lister Institute in London. It is pleasant to thank Messieurs Roux and Mesnil and Dr. C. J. Martin for their hospitality. They arranged demonstrations at which our preparations were shown to those in Paris and London who were most interested in them; and they gave us the use of their laboratories. We are especially indebted to the Pasteur Institute for supplying us with a number of reagents and the cultures of *Bacillus proteus X 19* used in Weil-Felix reactions.

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INTRODUCTION

THE general purposes of the Commission were to make etiological and pathological studies upon typhus.

The determination of the exact nature of the specific cause of the disease was held as the most important goal. A minute histo-pathological study was held to be an essential requirement for the understanding of typhus as a disease, and particularly for appraising relationships between lesions found and presumptive etiological agents which might be encountered. Bacteriological methods were deliberately given second importance, pending the development of indications during the research.

The transmission of typhus by the body or clothes louse was accepted as proved. The first information to be obtained was the nature of the demonstrable micro-organisms acquired by lice nurtured upon typhus patients. Subsequent work was to be directed towards the identification of a micro-organism in constant association with typhus in lice and finally, if possible, to prove by experimental means the identity of this micro-organism and the virus of typhus. Other evidence was to be sought for in the demonstration of micro-organisms in lesions specific for typhus and experience in Mexico (Wolbach and Todd, 1920) gave promise of success in this direction. Attempts at cultivation were to be deferred until we became convinced that strong proof was at hand in favor of a definite micro-organism being the cause of typhus.

The volume of work necessitated by the carrying out of this program on a sufficiently large scale, and the limited time at our disposal, made it impossible to attempt extensive serological or cultural observations. Nevertheless, thirteen unsuccessful attempts were made to cultivate the bacillus described by Plotz, Olitsky, and Baehr (1915), (see p. 113); and the

Weil-Felix reaction was employed and its diagnostic usefulness confirmed (see p. 33) in eighty-three cases.

Although the work of the Commission was planned independently, the results, both in regard to the etiology and pathology of typhus, prove in the main to be confirmatory of the work of many widely separated workers. We have added somewhat to the knowledge of the pathology and extended considerably the knowledge of the etiological agent. In general our work serves as a carefully conducted control to quite a number of researches made under less favorable circumstances in several countries.

For the study of a human disease transmitted by an invertebrate host two things are essential: a supply of patients infected with the disease to be studied, and an uninfected supply of the invertebrate hosts. The Commission had both at its command. The observations recorded in this report are based upon one hundred and eighty-one cases of clinically well-established typhus. The patients were selected from among general admissions to the St. Stanislaus Hospital, and were cared for in our wards. The lice employed in this study were taken to Warsaw from areas in North America and in Great Britain, where typhus is not endemic and were fed upon members of the Commission during the entire period of the research. Extensive control examinations of the lice used were made at the beginning and conclusion of the work.

II

TECHNIC

1. The Louse Stocks

The securing of stocks of lice free from demonstrable micro-organisms of any sort and beyond question free from the virus of any disease was of supreme importance. To ensure such a stock of lice, nits were obtained from a patient attending the Out-door Department of the Montreal General Hospital. These nits were hatched out on members of the expedition, Todd and Wolbach, and separate strains were maintained by each, known as the T and W strains. As may be seen in Table I (p. 7) the lice of these American stocks remained free from invasive micro-organisms of all types during the entire investigation.

A second stock of uninfected lice was brought by Mr. Bacot. These lice had been under observation and had been nurtured by Mr. Bacot since November, 1915. Lice from this stock were used for most of the trench fever researches by British and American commissions (Bacot, 1921, p. 156), and were proved to have been free from rickettsia-like organisms for over a period of two years prior to our research. Many lice from Mr. Bacot's stocks carried large gram-negative cocco-bacilli in the vagina and uterus or upon the surfaces of the copulatory apparatus of the male (folds of the vesica penis). Rarely, similar bacilli were encountered in the posterior portion of the alimentary tract. The uniform absence of bacteria from the intestinal canal of all lice is striking. These, or similar bacteria, were found in the uterus and vagina of but one female of the American stock. (See Arkwright and Bacot, "A Bacillary Infection of the Copulatory Apparatus of *Pediculus humanus,*" Parasitology, Vol. 13, No. 1, 1921, p. 25.)

Nearly the whole of the experimental work of the Commission was done with lice of the American stock, as early in the

research (April 17, 1920) Mr. Bacot developed an illness believed to be trench fever (Bacot, 1921, p. 156). The use of his stock was discontinued immediately after the onset of this illness in anticipation of the possibility of the lice becoming infected with some micro-organism. Mr. Bacot continued to feed his lice upon himself and after an appropriate period, eleventh day of his illness, rickettsia bodies began to appear in the lice excreta. Shortly afterwards large numbers of his lice proved to be heavily infected with rickettsia restricted to the alimentary tract and always extracellular. These rickettsia in distribution in the lice and in morphology were identical with *Rickettsia pediculi* of Munk and da Rocha-Lima (1917, p. 1424, Fig. 2), and likewise identical with *Rickettsia quintana* (Schmincke, 1917) (Munk and da Rocha-Lima, 1917, p. 1425), and *Rickettsia wolhynica* of Toepfer (1916). The last two names were applied to rickettsia bodies by the authors named in the belief that they were the cause of trench fever and distinct, though indistinguishable from *Rickettsia pediculi*. Arkwright, Bacot, and Duncan (1919[1], 1919[2]) and Byam and Lloyd (1919) described similar bodies in lice fed upon trench fever patients.

The symptoms and course of Mr. Bacot's illness were those of trench fever and the appearance in his lice of rickettsia indistinguishable from *Rickettsia pediculi* indicates the probable identity of *Rickettsia pediculi* with *Rickettsia quintana* (*wolhynica*).

2. Control Examinations of Stock Lice

Although none of the various members of the Commission who acted as hosts to the stock lice experienced any untoward symptoms, thereby suggesting that the lice were free from pathogenic micro-organisms, control examinations were made of each of the three stocks before our experiments were commenced. Serial sections and smears of the organs of lice from the three stocks were examined. These preparations were all stained by Giemsa's stain; the technic described below was used throughout our work.

At the conclusion of the research control examinations were again made. The results of these examinations are shown in the following table (Table I); they are uniformly negative except for Mr. Bacot's lice after the onset of his illness. Other evidence of the cleanliness of our American stock lice was furnished many times during the course of our work in the failure

TABLE I. CONTROL EXAMINATIONS OF STOCK LICE

	British		American			
	Bacot		Wolbach		Todd	
	Sections	Smears	Sections	Smears	Sections	Smears
First examinations, at onset of research	15—	57—	19—	40—	...	9—
Second examination, at conclusion of louse-feeding experiments	38 RE*	17— 45 RE*	26—	106—	...	11—
Totals.......	53	119	45	146	...	20
Grand total................................. 383						

* In this table, "RE" indicates extracellular rickettsia. Extracellular organisms, indistinguishable from *Rickettsia pediculi* or *Rickettsia quintana*, were found in Mr. Bacot's lice after he developed trench fever.

Intercellular rickettsia were found in none of these 383 lice.

to find micro-organisms in any louse of numerous boxes removed from typhus patients when conditions favorable to the development of *Rickettsia prowazeki* had not been maintained.

3. CONTROL EXAMINATIONS OF POLISH LICE

In order to ascertain if rickettsia occurred in lice found on apparently normal persons, lice were collected on three occasions from the garments of apparently healthy clients of a public bath-house in Warsaw. In all 148 lice were examined, 70 by serial sections, 78 by smears of the abdominal and thoracic organs. Rickettsia were found in twelve, in eight by smear preparations of the abdominal and thoracic organs, and in four by serial sections. The rickettsia in the serial sections were wholly extracellular. The rickettsia in the smear prepara-

tions were also indistinguishable from those associated with trench fever. (Repeated examination accounts for the slight difference between the figures given here and those mentioned in a preceding communication (Preliminary Report from the Typhus Research Commission of the League of Red Cross Societies to Poland, Wolbach, Todd, and Palfrey, 1920).

Since Mr. Bacot's illness developed fourteen days after working with these lice, we believe that his infection was probably contracted from them. His further observations (Bacot, 1921) prove that a person in good health may infect lice with rickettsia for at least three months after the cessation of febrile attacks or other obvious symptoms of trench fever.

4. The Feeding of Lice

The normal louse stocks and the lice fed upon patients were kept in cages (Fig. 1) substantially of the type described by Nuttall (1917, p. 106). A metal, not cardboard, box was used; the lower margin was flanged to facilitate the stretching and fastening of fabric. This flange at first was made by soldering a wire around the lower margin, but the spun flange is preferable, as it permits of rapid sterilization in the free flame. Bolting or milling cloth (to be obtained from dealers in millers' supplies) was used in place of chiffon as recommended by Nuttall. Bolting cloth is made of silk and is very strong and durable. We used cloth with 70 apertures to the inch; these are small enough to prevent the escape of larvae and large enough to permit all stages of the lice to feed freely. Narrow strips of adhesive plaster were used for fastening the bolting cloth to the bottom of the box. In wearing boxes containing the stock lice the straps devised by Nuttall proved useful (Figs. 2 and 3). To boxes designed for continuous wear we added a raised metal strap across the aperture in the cover under which the strap could be passed, thus removing any possibility of losing the box (Figs. 1, 2, and 3). In making up a cage for lice the border of the aperture in the bottom of the box should be pushed out slightly so that it presses against the bolting cloth which is drawn tightly over it.

Fig. 1. — Metal louse feeding box showing boiling cloth and metal cover.
Actual size

If this is not done lice, especially larvae and nymphs, will get between the bottom of the box and the bolting cloth and be destroyed. The edge of the aperture should be smoothed off to prevent cutting the cloth. Inside the box we placed a loosely fitting disc of thin black felt and, above it, a narrow strip of loosely coiled felt (any heavy cloth would do as well). After introducing the lice, bolting cloth is placed across the top of the box, the cover pressed into position, and the free edges of the bolting cloth trimmed with scissors. Finally, as a safeguard, the union of cover and bottom of box is sealed by wrapping with a strip of adhesive plaster.

(The boxes used by us were made from metal "tin" ointment boxes, 4 cm. in diameter, 1.5 cm. deep. Apertures 2.5 cm. in diameter were cut in the bottom and in the top of the cover. The leather straps (Figs. 2 and 3) used in wearing the boxes were 80 cm. long, in order that the boxes could be worn upon the leg).

In maintaining stock lice the boxes were worn daily for, at least, two periods of one hour each, separated by at least eight hours. Between feedings, the boxes were carried in waistcoat or trousers pockets for warmth. When it was desired to raise large numbers of lice the boxes were worn continuously. Overcrowding of stock boxes must be prevented by occasional removal of excess numbers of lice and the cast off skins (moults).

In badly soiled boxes the meshes of the bolting cloth may become clogged with excreta, a condition which requires transference of the colony to a freshly prepared box.

No difficulty was experienced in raising and maintaining our stocks of lice in these metal boxes, which have the great advantages of strength, durability, and ease of sterilization.

5. THE SECTIONING OF LICE

Our purposes demanded a technic which could be applied on a large scale and yet give the perfect fixation required for the demonstration of delicate micro-organisms in the tissues of lice. The obstacle to all stages in section technic — fixation, embedding, and cutting — is the impervious chitin cuticle.

It was found to be easier to dissect lice and to prepare them for embedding if their gastro-intestinal tracts were nearly empty. The absence of fresh blood from the stomach makes sectioning easier, as fixed fresh blood is very hard and brittle. For these reasons we starved all lice for 24 to 48 hours before dissection.

Very perfect results may of course be obtained with sections of organs removed from the louse; but it was desirable in our work to have the sections include all of the organs and their supporting structures *in situ*. The abdominal and thoracic organs of the louse can be removed with facility by the method described below, and, after arranging upon blocks of some homogeneous, previously hardened tissue, like liver or brain, may be fixed, embedded, and sectioned with ease. To fix the whole louse, penetration of the fixative must be ensured by cutting off the legs through the coxae as closely as possible to the thorax and by removing portions of the lateral margins on both sides of the abdomen. Both of these operations are best done in a trough of paraffin wax, to which lamp black has been added. A good binocular dissecting microscope is essential. In cutting off the lateral margins, which should be done after removal of the legs, extrusion of abdominal contents can usually be prevented if a very sharp knife is used and the body of the louse fitted into a small trench, carved in the wax, in such a manner that the lateral margin is supported by the edge. If the lateral margins are properly removed, enough of the musculature of the abdominal wall remains to prevent extrusion of the viscera.

Zenker's fluid, thoroughly saturated with corrosive sublimate, was the fixative used, and from six to twelve hours was allowed for fixation. Apparently, the length of time in the fixative did not influence the hardness of the chitinous parts. The process of dehydration is most important in influencing the ease of sectioning and the shorter the stay in alcohol, the less brittle becomes the chitin. As a rule, in less than two hours, the lice were run through graded alcohols into oil of cedarwood and were stored in this substance until embedded. Before em-

Fig. 2. — Louse feeding box applied as used in maintaining the stock lice for experimental purposes

bedding, the lice were placed for from one to several days in a mixture of about equal parts of paraffin wax and oil of cedarwood kept liquid at the temperature outside on top of the oven. For embedding, five changes of paraffin melting at 53°C. were employed, and three to five hours allowed.

Lice prepared and embedded by these procedures could be cut into serial sections, with a very small percentage of failures, at five to six microns.

6. DISSECTION FOR SMEAR PREPARATIONS OF LICE

Our endeavor in making smear preparations was to secure in each preparation *teased* rather than crushed tissues and to include all parts of the alimentary tract, the Malpighian tubules, reproductive glands, and the reniform and tubular salivary glands. For this purpose the dissection is best done upon a microscope slide clamped to the stage of the dissecting microscope.

A louse can be easily held in position upon a smooth glass surface by exerting slight pressure upon its back with a blunt needle or a pair of fine pointed forceps.

The first step in the dissection is to incise the thorax transversely on both sides, nearly severing it at the level of the first pair of legs, but leaving a narrow isthmus in the median line in order to avoid cutting the esophagus.

The second step is to place the points of the forceps upon the last abdominal segment; then, with a rolling motion compress the last two segments, push forward the contents and retain pressure upon the second or third segment. Now, the last segment and a half should be cut off and, with the forceps still in position to prevent protrusion of the viscera, the head and anterior end of the thorax with the first pair of legs is torn off by traction with the back of a small knife or a sharpened triangular shaped needle; the traction should stop before the esophagus is torn.

The third step is facilitated by placing a drop of salt solution upon the louse. It consists in alternately wiping forwards with the flat of a flexible knife blade from the point of pressure

upon the posterior end of the abdomen and exerting traction upon the head. Instead of wiping with a flat flexible instrument, the abdominal contents may be expressed by rolling forwards under pressure with a needle or fine glass rod. By this means it is possible to eviscerate rapidly the thorax and abdomen and obtain intact in one piece the whole of the alimentary tract, the Malpighian tubules and salivary glands of both types. With care the reproductive organs can also be expressed forwards with little or no injury. (For a description of the anatomy of the human louse see Sikora, 1916, Beihefte 1, Archiv. f. Schiffs- und Tropenhygiene.)

Between dissections of lice it is imperative to clean with care the instruments so that organisms which are present in one louse are not introduced in the preparations of succeeding lice. When working with typhus infected lice several sets of instruments are advisable so that each set may be sterilized in phenol or lysol after using and before washing.

In handling boxes containing infected lice, louse-proof gowns and rubber gloves were worn. The excreta were regarded as infective and were permitted to fall only upon cloths wet with disinfectants. The boxes were opened in large glass crystalliz-ing dishes while under observation by a second person. The lice were removed to glass weighing bottles with the aid of a Zeiss prism magnifying binocular and a count was kept of all saved for dissection. Discarded boxes with possible overlooked immature stages and eggs were immediately placed into a sterilizer.

In handling lice we have found the prism magnifying bin-oculars indispensable, and have always used very finely pointed yet stout forceps for grasping them by a leg seized close to the body (femur).

7. Staining Methods

Smear preparations were air dried, fixed for fifteen to twenty minutes in absolute alcohol and stained for a period of three hours or longer in Giemsa's stain diluted in proportions of one drop to one cubic centimeter in distilled water.

FIG. 3. — Strap and louse feeding box adapted from Nuttall (1917, p. 106)

The formula for the Giemsa stain is: Azur II eosine (Grübler), 3 grams; Azur II (Grübler), 0.8 gram; acetone free methyl alcohol, preferably Merck's reagent, 375 grams; Merck reagent glycerine, 125 grams.

Sections of lice and sections of human and animal tissues were stained by the modification of Giemsa's method described by one of us in work on Rocky Mountain Spotted Fever (Wolbach, 1919).

Louse and mammalian tissues were fixed in Zenker's fluid saturated with corrosive sublimate and with the addition of 5 per cent of glacial acetic acid. The acetic acid was used in order to prevent the staining of mitochondria, although slightly better results may be obtained without it. While we do not believe that the presence of stained mitochondria would be a source of error we preferred to obviate one source of query in the discussion of results.

After embedding in paraffin and sectioning, the slides were treated by the usual methods except that an extra step was taken to insure complete removal of the iodin employed to remove corrosive sublimate crystals deposited in the tissues. After the iodin, the sections were treated with 0.5 per cent solution of sodium hyposulphite for ten to fifteen minutes, and then thoroughly washed in running water followed by distilled water.

The sections were stained in a slightly alkaline mixture. The distilled water used in the solution employed for staining sections should be free from acid to begin with and will have the right reaction after the addition of from two to four drops of 0.5 per cent solution of sodium bicarbonate to one hundred cubic centimeters of water. It may be necessary to determine the exact amount of alkali by trial. The addition of methyl alcohol to the staining solutions, three to four cubic centimeters to each hundred cubic centimeters of water, retards the precipitation of the dye and yields more intense staining.

The formula for the stain which has given the most uniformly good results is: Distilled water, 100 cubic centimeters; 0.5 per cent sodium bicarbonate, two to four drops; reagent methyl

alcohol, 3 cubic centimeters; Giemsa's stain, 2.5 cubic centimeters.

The stain should be poured over the slides immediately after mixing, and should be changed twice during the first hour, and allowed to remain in the third solution for twelve to eighteen hours. The slides, which are heavily overstained by this method, are differentiated in 95 per cent ethyl alcohol. The procedure of differentiation really consists in removing the excess of stain to a point where good histological detail is secured. If the sections are too blue, a better balance may be secured by adding very small quantities of colophonium to the alcohol. After differentiation the sections should be rapidly dehydrated in absolute alcohol, cleared in xylol and mounted in oil of cedarwood.

The various steps in the technic are as follows:

1. Fix in Zenker's fluid (formula: corrosive sublimate, 6 grams, potassium bichromate 2.5 grams). Twenty-four hours should be allowed for animal tissues, but two to six hours for louse tissues is sufficient.

2. Embed in paraffin and section at 6μ or less.

3. Xylol, alcohol, Lugol's solution; alcohol as usual.

4. 0.5 per cent sodium hyposulphite to remove the last traces of iodin, ten to fifteen minutes.

5. Wash in running water ten minutes, followed by distilled water.

6. Stain, differentiate, dehydrate and clear as above, and mount in oil of cedarwood.

The staining of paraffin sections by Giemsa's stain is no more complicated or difficult than using the eosin-methylene blue stain. One condition, however, is absolutely essential, and that is that the sections should be thin, not over six microns.

III

CLINICAL OBSERVATIONS

1. Methods, Character of Material

THE clinical cases upon which the studies of the Commission were based were found at the St. Stanislaus Hospital, Warsaw, Poland. There, an arrangement was made by which selected patients were transferred for the active course of their disease to a division of the hospital which was entirely under the control of the Commission. The St. Stanislaus Hospital was the chief hospital in Warsaw for typhus fever patients. To it were sent cases diagnosed as typhus by the health officers of the city. No cases were recommended to it except those believed to have typhus. As a precaution against error, however, all cases admitted were retained in receiving wards until the officer of the St. Stanislaus Hospital Staff in charge of these wards was satisfied that the diagnosis was correct. When the diagnosis was thus confirmed patients were transferred to the general typhus wards. It was from patients thus admitted to the general wards with confirmed diagnosis that cases were selected by the Clinical Officer of the Commission, in consultation with one or more members of the St. Stanislaus Hospital Staff, for transfer to our Division. All cases admitted to our Division, therefore, were cases in which the diagnosis of typhus fever was considered unquestionable as the result of not less than three examinations by four or more physicians familiar with the disease.

On admission to our Division, after a careful delousing process, an independent history was taken and a complete physical examination was made. As a routine the urine was examined, and the percentage of hemoglobin and number of white cells in the blood was ascertained. In many cases, there was done also a differential count of the white cells, an examination of smears for the organisms of relapsing fever and mala-

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ria, serum reactions for typhoid and paratyphoid A and B agglutinations, and a Weil-Felix reaction. Four-hourly charts of temperature, pulse, and respiration were kept by our nurses. Each case was visited and examined daily by the Clinical Officers and a daily note of progress was recorded. Patients were retained in our Division only until it was concluded that they had passed the active stage of typhus fever, when they were returned to their original wards to complete their convalescence. In fatal cases arrangements were made to hold autopsies very shortly after death.

The group of 181 cases studied is a selection of the most typical of the severer cases received by the hospital. Our cases were chosen first for certainty of diagnosis, second for gravity of prognosis, and third for early recognition in order that the louse-feeding experiments might include early as well as late cases. Owing to the well-known fact that the mortality in typhus fever is higher in older patients, age was often a factor in our decisions as to which patients should be transferred to our wards. Our material, therefore, differed from that of the remainder of the hospital in the relatively small number of young and mild cases. We were unable, however, to accept all of the severe cases, so that certain patients died and became available for autopsy who had not been transferred to our care in life. Still, in his rounds for the selection of cases the Chief Clinical Officer of the Commission was able to see practically all of the important clinical material of all wards of the hospital. Of the fourteen cases coming to autopsy not from our Division he had seen and examined eight sufficiently to vouch for the diagnosis on clinical grounds. In the remaining six cases the clinical diagnoses of members of the St. Stanislaus Hospital Staff is accepted.

From the selected clinical material gathered in this way it was possible to obtain facts as to the course of the disease in each case; they are here published for their descriptive value. In connection with the data obtained from history, it is necessary to explain that the difficulties of communication resulting from ignorance and delirium of many patients, as well as the

necessity for interpretation of their language, may well have resulted in inaccuracies in certain instances. It was often only after much questioning that the duration of illness before arrival in our Division could be determined even though the symptoms of onset were well described and seemed to have appeared abruptly. Some instances of apparently abnormally long or abnormally short courses may have been due to error in statements of duration on admission. In general, however, the facts were obtained as accurately as careful questioning could effect, and it is probable that in the combined statistics such errors as were unavoidable largely offset one another.

2. MORTALITY

It may also be mentioned that, as will be seen from the statistics, the epidemic with which we were dealing was one of the disease in a mild form. The mortality among the recent total admissions of the St. Stanislaus Hospital and of other typhus hospitals in the neighborhood was estimated as not over 7 per cent. The mortality of 13.26 per cent of our cases, even though they were selected largely for signs of dubious prognosis, is low as compared with the mortality of many typhus fever epidemics.

3. INCIDENCE

Our series of cases included 181 patients, 86 men and 95 women. The great majority were residents of the city of Warsaw although some were refugees from a distance. Nearly all were from the lower classes; many were Hebrews. There were frequent instances of infection of two or more members of the same family.

The ages are not typical of the age-incidence of the disease because we selected older patients for our work. The increasing mortality with advancing age is well illustrated in the table of deaths (see p. 32) for each decade.

4. PRODROMATA AND ONSET

Attention was devoted to the question of the existence of a prodromal stage of typhus fever. On close questioning a large majority described some symptoms, never prostrating but rather to be classed as an ill-defined malaise for from one to three or more days before the onset of severe symptoms. Our findings tend to strengthen the belief that a prodromal stage exists, but that its symptoms, in the absence of known exposure or other suspicious circumstances, are too vague to be of diagnostic value.

The true onset, in contrast, was in nearly all cases sharply defined. On a certain day and even at a certain time on that day the patient realized that he was sick and as a rule had to give in to the disease at once. On admission to our service he often had difficulty in remembering the number of days that had elapsed since this invasion, but its circumstances were ordinarily well described. Patients too delirious on admission to give a history were able to remember the symptoms of invasion when they became convalescent, although the remainder of the course of the disease was often a period of oblivion.

The commonest symptom of onset was headache in 161 cases. It was described as very severe and was frontal, occiputal or general. It was often the chief source of discomfort for several days, but had diminished or ceased before the stage of eruption appeared.

Chill was second to headache in its prominence, occurring in 134 cases. In 84 of these cases there were two or more chills in the first day or days of illness. The remaining 50 had only a single chill.

Pain in the back and limbs was a complaint in 97 cases but was in most of these subordinate to the headache. Vomiting occurred with onset in 53 cases. Constipation was the rule in the early stages of the disease, being present in 157. Diarrhoea was rare, figuring more in the later stages as will be mentioned under progress. Anorexia was an early symptom in but 77, the

remainder retaining their appetite, some to a degree unusual in fevers. Insomnia was complained of in 88 cases.

Most of our patients were not admitted to our Division until on or after the sixth day of illness. Through poverty, ignorance, and other difficulties in securing medical attention they had as a rule remained some days sick at home before they were seen and sent to the hospital by a district health officer of the city. It was usually on the day following their admission to the hospital that they were selected for transfer to our service. Most commonly the eruption was present in its earlier stages on admission to the hospital. An unquestionable eruption was required by us in all cases accepted for transfer. Diagnosis by the eruption was ordinarily clear and simple with the exception that the lesions were in not a few cases complicated and obscured by those of chronic pediculosis and impetigo. Some cases undoubtedly having typhus fever were rejected on this account.

All cases entering our Division did so when they were febrile and had eruptions of the types described below. On entering 70 patients showed outspoken mental disturbance, 45 had delirium of the excited type, and 25 mental dullness; the remainder did not show obvious mental symptoms, but many of them developed delirium later, often on the first evening.

The facies on admission was noted as "tense" in 49 cases, corresponding commonly with excitement, and "lax" in 23, corresponding with dullness or stupor. The face was markedly flushed in 73.

Conjunctival injection was noted on admission in 157; in 83 cases moderate, in 68 marked, and in 6 extreme. The conjunctival injection, like the delirium, tended to increase with the progress of the eruptive stage until the beginning of defervescence.

The tongue was noted as coated but moist in 86, coated and somewhat dry in 48, and very dry in 26. Dryness of the tongue was regarded by the Polish physicians as one of the most reliable criteria of the severity of a case, and our experience taught us to concur in this opinion. In all severe cases the tongue and

mouth became dry and fissured in spite of constant care. There was moderate reddening of the pillars of the fauces in 59 cases but never of such intensity as to suggest marked inflammation.

Labial herpes was present in but two cases.

5. ERUPTION (Figs. 4, 5, 6, 7, and 8)

All on admission showed an eruption characteristic of typhus fever. This eruption is in its early stages composed of discrete, rather sharply defined, pink macules, round, oval, or somewhat irregular from 2 to 6 mm. in diameter, disappearing on pressure, rarely palpable, distributed with more or less profusion (but nearly always more numerously than the rose spots of typhoid fever) on the chest, abdomen, back, neck, shoulders, arms and legs, and dorsa of hands and feet,— in other words over the whole body with the exception of the face and head. In our cases on first examination it was present on the trunk in 180, one case having too extensive pigmentations and scratch marks from pediculosis for recognition of the typhus rash except on the extremities. One hundred and forty-two cases at this stage had eruption on the extremities, 101 on the neck. As curiosities 6 cases with profuse general eruptions had a few lesions on the face, 2 had lesions on the palms, and 2 had red spots probably typhus lesions on the soft palate. As the disease progresses the lesions become more numerous and larger, and the color becomes first a brighter red, then purplish red to dark purple. As the purplish color develops the lesions often tend not to disappear on pressure, due to extravasation of blood, but the hemorrhagic element in the eruption is commonly less than the color of the lesions would lead one to suspect. Even the deepest purple lesions may disappear completely on pressure, and some of our patients who up to the time of death had had extreme eruptions showed almost complete disappearance of eruption after death. Among our cases the rash as tested by disappearance on pressure was markedly hemorrhagic in 52. In 14 some lesions were palpable. In some cases somewhat after the beginning of the stage of eruption,

FIG. 4

Fig. 5

Figs. 4 and 5. — Side and back of a patient showing the distribution of the rash. Second week of typhus

Fig. 6. — From the same patient shown in Figs. 5 and 6, showing details of the rash. The dark colored areas are petechiae, the lighter colored have discrete areas disappeared upon pressure

FIG. 7. — Erythematous and petechial eruption. Second week of typhus

Fig. 8. — Erythematous and petechial eruption. Second week of typhus

especially in young subjects with clear skins running high temperatures, the eruption, particularly on the neck, chest, and upper arms, is complicated by a mottled or blotchy erythema which may continue for several days but disappears earlier than the lesions of the typical eruption. This was observed in 23 of our cases.

On direct examination of the skin of a typhus patient with a microscope magnifying 40 diameters, first rubbing on petrolatum to increase transparency, each lesion is seen to consist of a tangle of dark red blood vessels.

6. Lungs and Heart

Among our cases evidence of tracheobronchitis was so prevalent as to lead us to believe it characteristic of the disease as with measles. On transfer to our service nearly all cases had cough, either dry and slightly barking or loose with obvious excess of bronchial secretion. In 55 this cough was present on admission without abnormal signs in the lungs. Fifty-five showed musical râles in varying numbers throughout. Seventy-three showed crepitant râles at one or both bases.

The heart showed no evidence of endocarditis in any instance. In 8 cases the heart was absolutely irregular without visible auricular venous pulse in the neck as from auricular fibrillation. In the 4 of these cases that recovered the heart resumed normal rhythm with convalescence; the remaining 4 died, but without symptoms or signs of cardiac insufficiency.

7. The Spleen

The spleen was palpable on admission in 48 cases.

8. Blood

White counts were done by Dr. Stella Naparalska on the day following admission in 179 cases. The results are shown in the following table (Table II).

Certain cases with high leucocytoses, however, recovered without at any time in their course showing signs of a severe type of typhus infection or of complications.

In 59 cases Dr. Monroe A. McIver examined blood smears stained by Giemsa. Special attention was given to the forms of leucocytes and differential counts were made. He studied particularly the relative proportion of neutrophiles with no division of the nucleus to those with two or more divisions connected only by threads. This investigation was done because it had been reported that in typhus fever an abnormal number of the neutrophiles have undivided nuclei, and that this observation had diagnostic value.

A composite differential count constructed by averaging the totals of these 59 counts, all taken in the second week of the

TABLE II

Number of White Cells	Cases	Number of Deaths	Mortality
6– 8,000	12	2	16.6 %
8–10,000	20		
10–12,000	66	12	9.2 %
12–14,000	44		
14–16,000	22		
16–18,000	8	8	21.6 %
18–20,000	7		
Blood counts omitted	2	2	
Totals	181	24	13.26 %

disease, is as follows: Lymphocytes, 18.6 per cent; large mononuclear basophiles, 5.9 per cent; neutrophiles, 75.4 per cent; (neutrophiles with incompletely divided nuclei, 50.7 per cent; neutrophiles with two or more distinct divisions of the nucleus, 24.7 per cent). In two cases two cells were seen in the course of the count in each which had all appearances of myelocytes. Eosinophiles were completely absent in all smears, as is known to be characteristic of typhus fever. Blood platelets were apparently normal in number but seemed to be of unusually large size. The red cells were of normal appearance.

Finger blood from 169 patients was examined, usually once only, for rickettsia. The blood was taken at all periods of the disease, usually within the first ten days. Thin and thick

smears were taken from each case. Thick smears were de-hemoglobinized before staining; both thick and thin smears were fixed with absolute alcohol and stained with Giemsa. Granules of various sorts were seen and bacteria, usually coming from water or stain, were frequently found. In no instance were appearances observed which could with any approach to certainty be regarded as rickettsia; nevertheless, appearances were seen in two or three cases of which it could not be said that they were not rickettsia. No cases showed plasmodia of malaria or organisms of relapsing fever.

9. URINE

In all cases the urine was concentrated, with albumin in slight to moderate amounts, as in other febrile diseases of like severity. No case showed findings in the urine which could warrant a diagnosis of acute nephritis.

10. AGGLUTINATION REACTIONS

Mr. Henry Pinkerton, S.B., of the Commission did agglutination tests for *B. typhosus, B. paratyphosus A* and *B. paratyphosus B* with the blood serum of 90 of our cases (see p. 34). *B. typhosus* was agglutinated in maximum dilutions of from 1–200 to 1–1600 in 15 cases; in maximum dilutions of 1–25 to 1–100 in 27; 48 gave no agglutination. One of the cases of high agglutination gave a history of previous typhoid; two others, and perhaps more, had probably had immunizing injections. But in most cases of positive agglutinations with the typhoid bacillus no clinical explanation was found, either in the past history or in signs of double infection in the current illness. It is probable that these agglutinations were due to the peculiar propensity of typhus fever to show non-specific agglutinations. The reactions with the paratyphoid bacilli, both *A* and *B*, however, were all negative with the exceptions of the two cases believed to have had prophylactic injections.

Mr. Pinkerton also made agglutination tests with two strains of *Bacillus proteus* (the Weil-Felix reaction) in 83 cases. His

results which are tabulated (Table VII, pp. 37, 38, and 39) corroborate the estimate of other workers in regard to the certainty and value of this test.

11. Clinical Course

In the progress of the cases in our wards after admission the two outstanding symptoms aside from the usual manifestations of continued fever were delirium and cough. Delirium was a marked feature in 86 cases, and undoubtedly existed though in a less demonstrable form in more cases than could be recognized, since not a few patients who had been quiet and apparently rational through their illness later had no memory of having been in our wards. In general the delirium was of a peculiarly active type, noisy and violent, with constant attempts to get out of bed and with violent resistance to all nursing ministrations. It seemed to increase in severity from the time of admission early in the eruptive stage until the beginning of defervescence when in favorable cases it diminished rapidly, and in certain unfavorable cases it gradually passed into a partial or almost complete coma to be described later.

Cough was troublesome and exhausting in 69 cases. Except in the earlier stages it was accompanied by profuse mucopurulent expectoration. Hoarseness or aphonia was frequent. As the disease progressed the musical râles present in a large proportion on first examination tended to disappear, while crepitant râles especially at the bases became commoner. At some time in the disease these coarse moist râles were present at one or both bases in 144 cases, and often were more or less widely distributed through both lungs. Local signs of consolidation, dullness with bronchovesicular or bronchial breathing and whisper, occurred in 16 of the cases that recovered. These are believed to have had confluent bronchopneumonias with the exception of one who developed a typical lobar pneumonia immediately after the defervescence of a severe typhus. Terminal bronchopneumonic symptoms, with or without demonstrable consolidation, occurred in 18 fatal cases.

In severe cases of typhus fever the delirium of the earlier
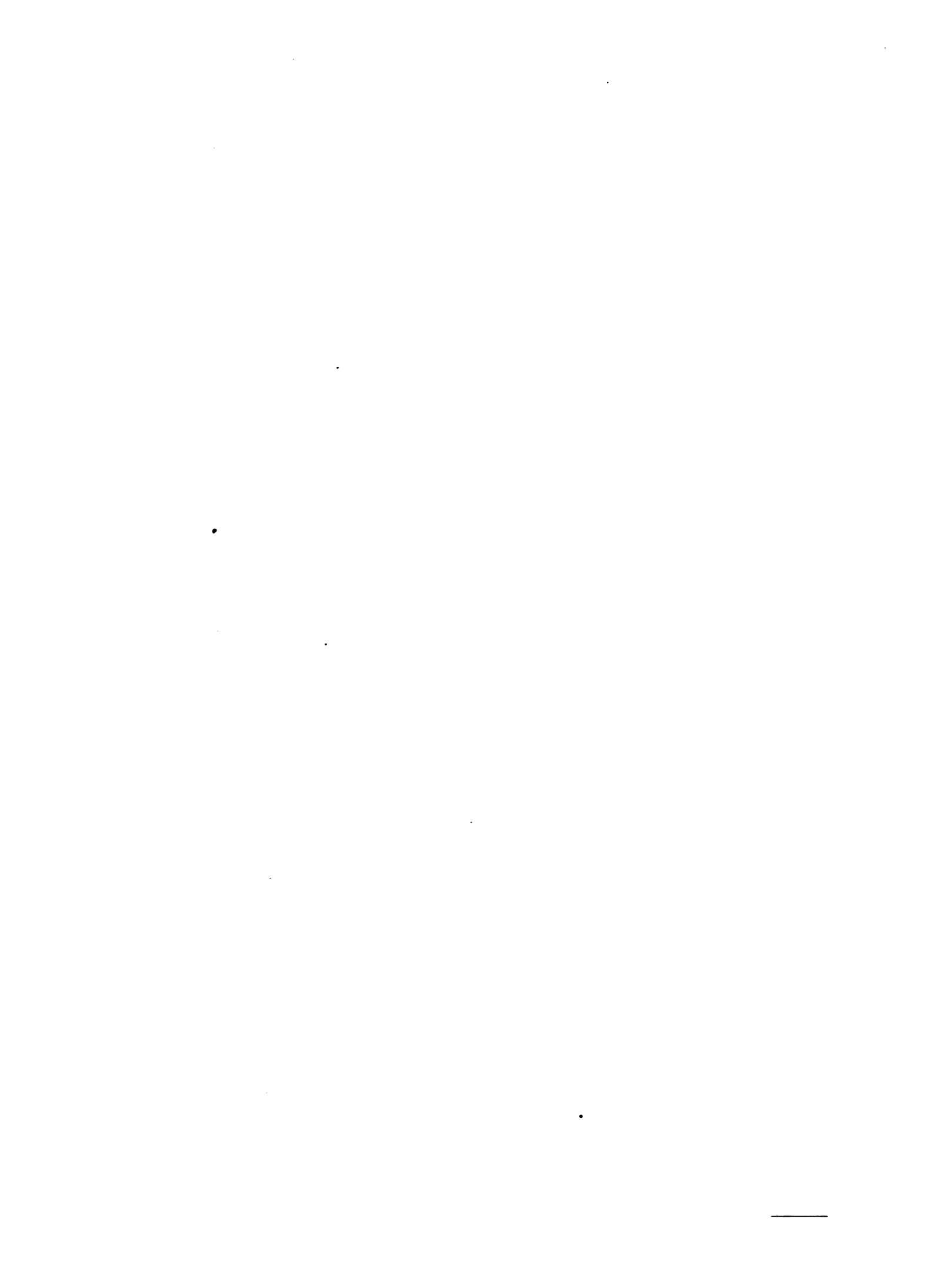
FIG. 9. — Facies of typhus; patient emaciated and in coma

stages is often replaced as the disease progresses by a stupor which increases to almost complete coma and persists in spite of defervescence until death occurs sometimes many days after the fall of temperature. This syndrome was encountered in 11 of our cases. In spite of rather early defervescence the mental and general condition of these patients continued to become progressively worse. Nutrition became increasingly difficult and emaciation became marked except in those previously obese (Fig. 9). The eruptions in these cases persisted without fading in spite of normal temperatures until death. After a week, more or less, of this coma with little or no fever terminal rises of temperature with rapid labored breathing supervened and the patients died with the clinical appearance of terminal bronchopneumonia as in other exhausting diseases. Two cases had convulsions immediately before death. Meningismus was more or less marked in the cases of coma. The secretion of urine was not suppressed nor was there other evidence of nephritis.

Gangrene of the skin and underlying structures occurred in 6 of our cases (Figs. 10, 11, and 12). Thrombosis of the right iliac artery was present in 1 case. Otitis media occurred in 4 cases. Marked deafness persisting with gradual improvement into convalescence without evidence of middle ear involvement was present in 5. Erysipelas appeared in 1 case. Phlegmons of the skin in 3 are noted. Parotitis occurred in 7 cases and submaxillary gland infection in 3. These infections of salivary glands were all suppurative with 1 exception but were accompanied by less severe general symptoms than are common in suppurative parotitis in other diseases. All recovered after incision except 2 in which the gland inflammation appeared only when the patients were already moribund, and in which the adenitis is not believed to have contributed materially to the fatal result.

Constipation was the rule throughout although diarrhoea was present in 17 cases.

The clinical course of typhus fever as illustrated by our graphic charts of temperature, pulse, and respiration shows

such variations that description and analysis are difficult
(Temperature charts 1, 2, 3, and 4). Some cases would show
continuous high temperatures with less other evidence of seri-
ous illness than would be expected. Other cases would present
the gravest clinical picture, often ending in death, with only
moderate temperatures. From a general survey of our charts,
however, impressions are gained which may be summarized as
follows. Through the obscuring confusion of variations it is
thought that a normal curve of temperature for uncomplicated
typhus ending in recovery can be made out, represented by a
continuously elevated temperature in the neighborhood of
103°F., with or without slight to moderate remissions, most
commonly in the morning, and returns to the higher level in the
afternoon; this fever continuing at a fairly constant average
height, or showing a slight downward tendency in the second
week, until most commonly on the morning of the 11th or 12th
day a marked remission of temperature, almost or quite to
normal, occurs accompanied by a distinct improvement in the
general condition. The temperature rises in the next afternoon,
and on the following day the remission and evening rise are
repeated. But a third remission, occurring forty-eight hours
after the first, very commonly marks the beginning of per-
manently normal temperature, although still another evening
rise may appear. We suspect that it is the conspicuous im-
provement in the general condition, often accompanying the
first remission, which has led to the common teaching that
typhus fever is a disease which terminates by crisis. As the
curve of the temperature shows, the defervescence of typhus
fever is better described as rapid lysis (Temperature charts 1
and 2). The chart described above, it must be admitted, is so
subject to variations that fully typical instances are the excep-
tion. Many of our cases during their course had occasional un-
accountable marked remissions in single readings of tempera-
ture without corresponding improvement. Others ran a more
or less regularly remittent temperature throughout the later
part of their course somewhat simulating the later stages of
typhoid fever. Others ran an absolutely irregular temperature

FIG. 11. — Necrosis of skin and subcutaneous fat from cold. Operation and skin-grafting of the limbs healed. The upper limb is an example where drug reaction from therapy was responsible for the gangrene.

FIG. 11. — Necrosis of skin over tuberosity of ischium, internal condyle and olecranon process of ulna

Fig. 12. — Necrosis of skin, subcutaneous fat and muscles of buttock and heels

curve. In a few cases the defervescence took place by a continuous fall within twenty-four hours or less. Others showed a gradual lysis without any distinct remission or other sign of outspoken improvement on any single day. Still in going over our series of charts and records it seems to us that most cases represent only variations in rather than contradiction of this curve suggested as the normal. After defervescence with subsidence of typhus symptoms most cases showed continuously normal or subnormal temperatures; others, however, had evening rises for some days without notable febrile symptoms, probably from persisting bronchitis. In certain severe cases, and notably in those cases passing from delirium into stupor ending in death, the elevation of temperature was conspicuously slight. It deserves emphasis that in typhus fever, more than in any other acute infectious disease, the temperature may bear little relation to the severity of the general symptoms. Among the fatal cases there were several instances of terminal hyperpyrexia.

The pulse curve through the febrile course of many of our cases was characterized by great variability of rate. Certain cases day after day would show rises and falls as great as between 80° and 140° (Temperature charts 2 and 4). These variations followed the temperature curve or were dissociated from variations in temperature. They were rather apparently associated with increases or remissions in the delirium. In cases with a more constant pulse rate the pulse was rapid in proportion to the temperature, in contrast to typhoid fever. Dicrotism, however, was not infrequent. In severe cases with cold cyanotic extremities the radial pulse often could not be counted for days. With the onset of a complicating pneumonia the acceleration and weakening of the pulse accompanied the appearance of pneumonic dyspnoea (Temperature chart 3). With defervescence and beginning convalescence the pulse curve followed a striking downward course, the slowing often being clearly evident from the first remission of temperature in anticipation of the final disappearance of fever. Bradycardia of convalescence was sometimes marked.

The blood pressure in a series of 23 cases on which daily determinations were made during their period in our wards was in most cases abnormally low. Systolic pressures under 80° and diastolic pressures under 50° occurred in several cases. The pressures from day to day changed little, but in most there was a slight rise with defervescence often followed by a slight depression in early convalescence. Our observations did not tend to confirm the theory that low blood pressure is an important factor in unfavorable prognosis, since in the three fatal cases in this series the blood pressure was higher than the average in all. The number of cases, however, is too small to warrant general conclusions.

The respiration curve showed less irregularity of rate than the pulse curve. Most cases showed moderate to marked acceleration through the febrile period. The graphic curve, however, fails to show the true state of the respiration. Many cases of uncomplicated typhus would breathe rapidly with gentle shallow respiratory movements, without dyspnoea or discomfort. When, however, bronchopneumonia supervened the dyspnoea rapidly became urgent although the rate as recorded on the chart showed no such conspicuous change as was evident from looking at the patient.

The duration of fever in cases that recovered, according to the best information that we could obtain as to time elapsed before admission, was commonly about two weeks. We estimated that defervescence occurred by days of disease as follows: 9th day, 1 case; 10th day, 5 cases; 11th day, 12 cases; 12th day, 13 cases; 13th day, 25 cases; 14th day, 42 cases; 15th day, 27 cases; 16th day, 14 cases; 17th day, 10 cases; 18th day, 3 cases; 19th day, 2 cases; 20th, 21st, and 22d days, 1 case each. Error in histories may account for some of these variations, but in general the early defervescences occurred in mild cases, particularly in younger patients, while the long courses were commonly severe ones.

Deaths occurred on days of disease as follows: 7th day, 1; 8th day, 2; 10th day, 1; 11th day, 5; 12th day, 3; 13th day, 1; 14th day, 3; 15th day, 2; 16th, 17th, 18th, 21st, 24th, and 27th
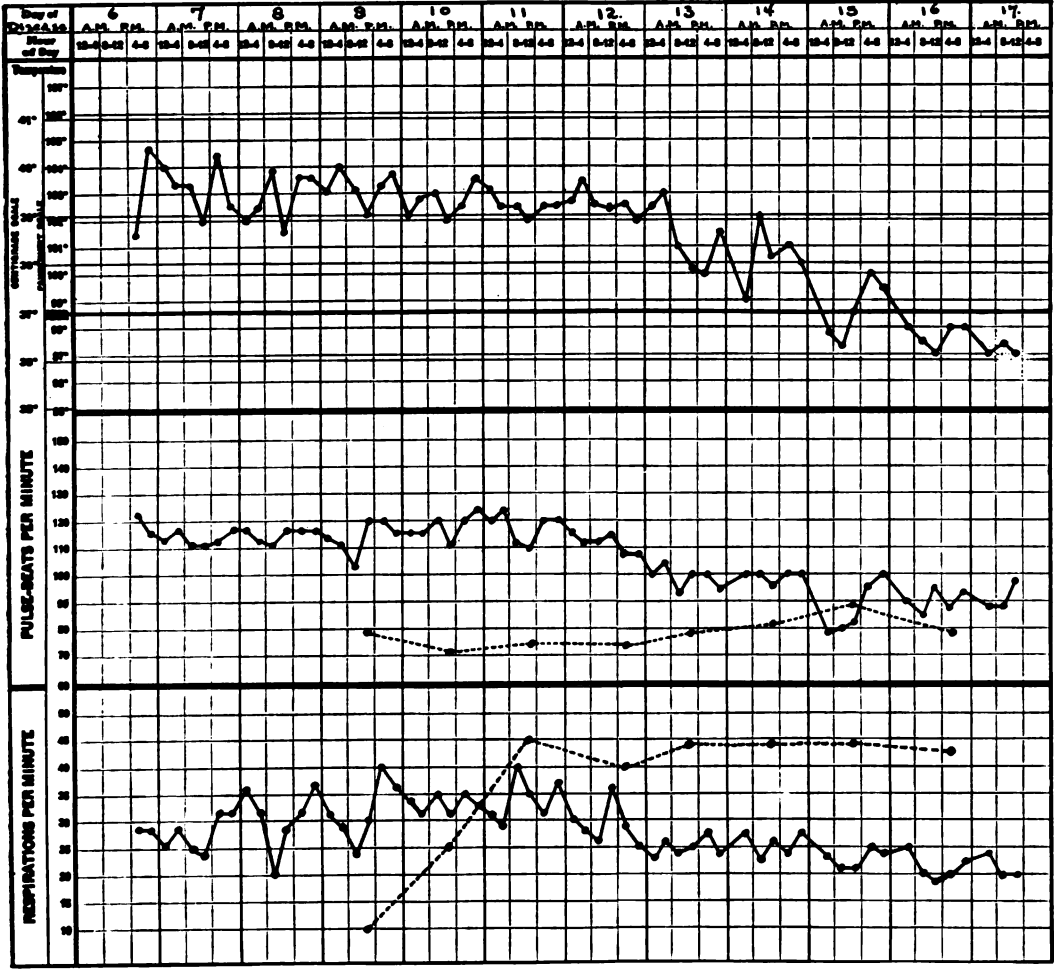
CHART 1. — A typical case of uncomplicated typhus fever in a young patient, with recovery
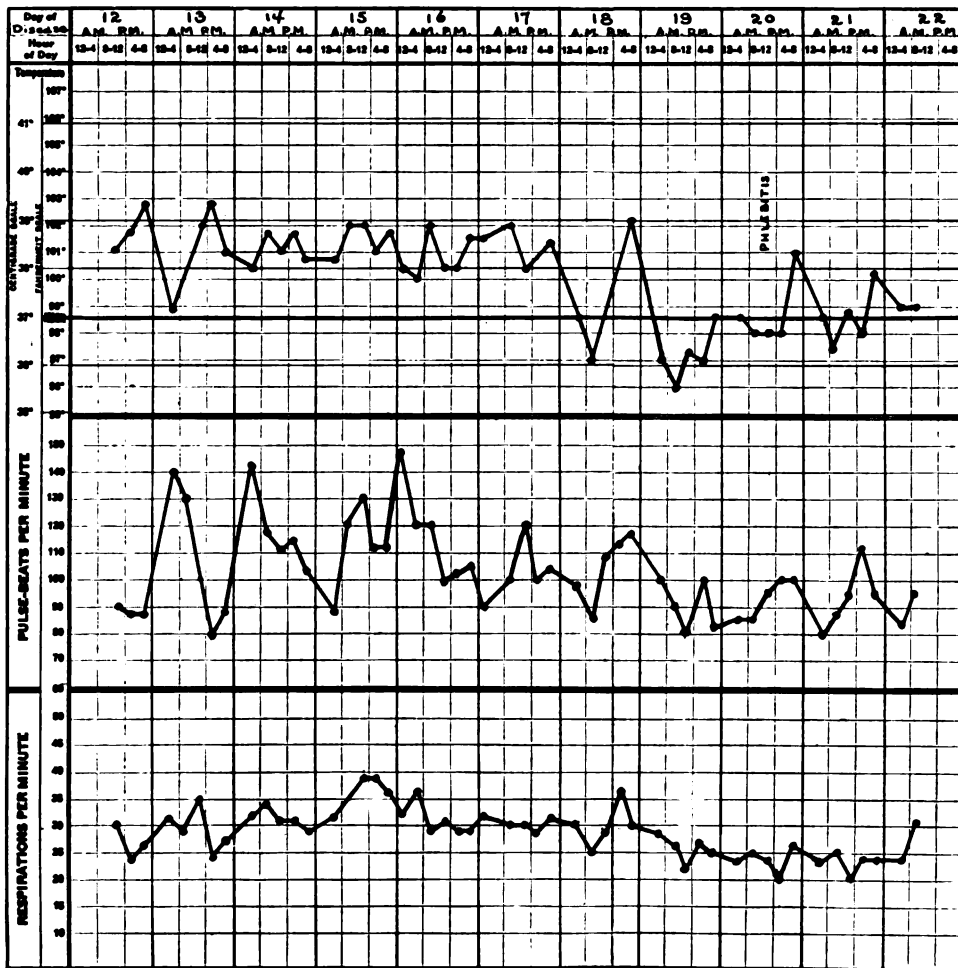
CHART 2. — Moderately severe typhus with marked delirium; recovery. Illustrating the
moderate degree of fever and the variability of the pulse rate
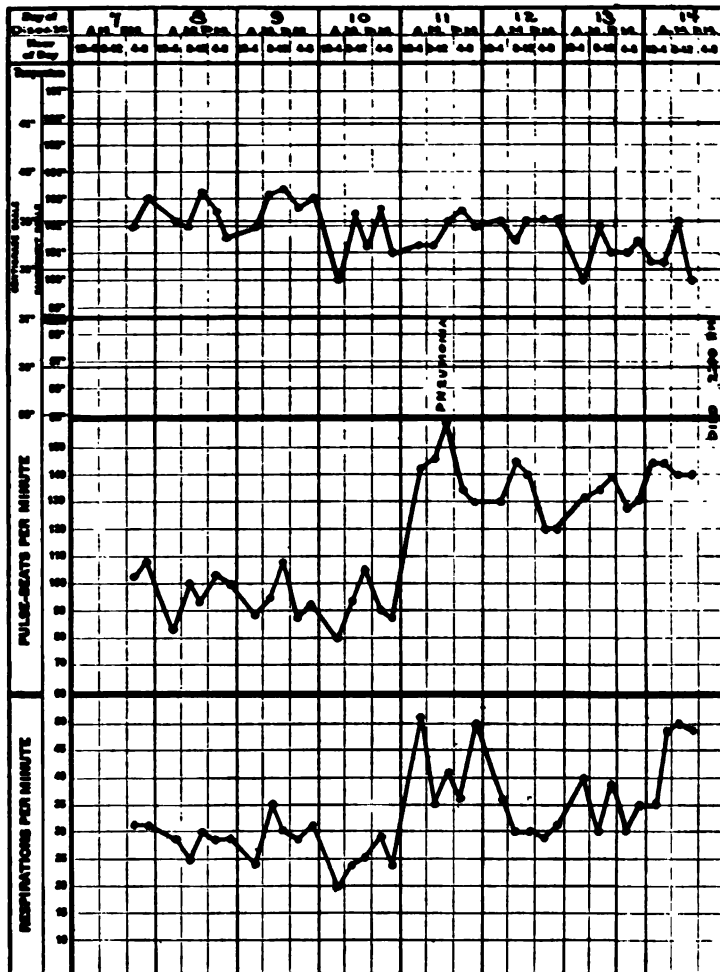
CHART 3. — A case complicated by fatal bronchopneumonia
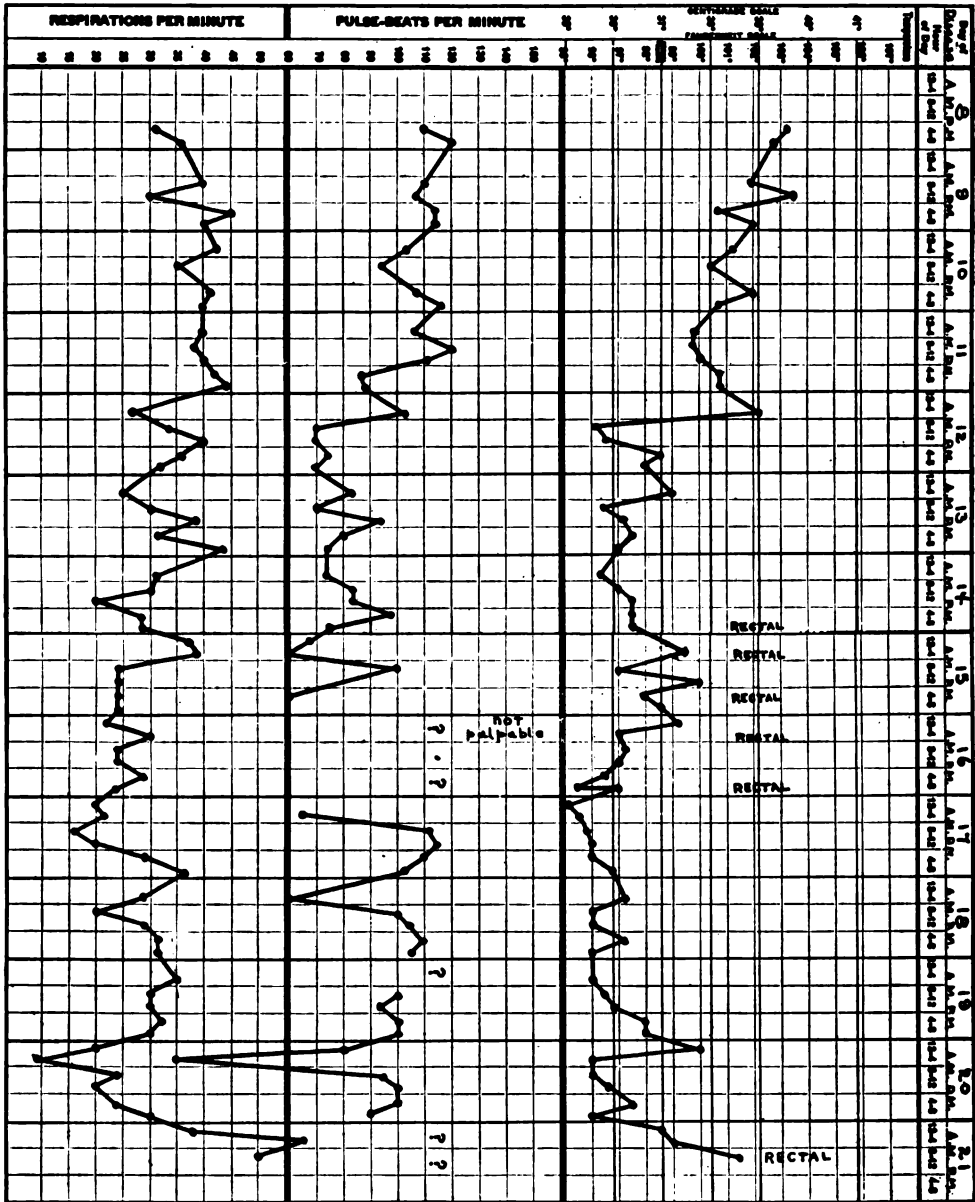
CHART 4. — A fatal case, after a mild early course, passing into prolonged coma with terminal bronchopneumonia.

days, 1 each. The deaths in the later days followed periods of stupor with little or no fever but with persistent eruption.

12. TREATMENT

Our treatment consisted in a routine planned to provide for rest and comfort so far as possible with supporting and symptomatic measures. The chlorine injection treatment described by Danielopolu (1919, p. 335) had not come to our attention. Although preparations were made to try the therapeutic value of the serum of convalescents in larger doses than have been employed by previous investigators these could not be completed before it was necessary to depart.

All cases admitted to our Division were submitted to a second delousing process more complete than that practiced on admission to the hospital. To this we attribute the non-occurrence of typhus in workers and visitors in our wards. Each patient was stripped on a stretcher on which he was brought to the Division and all clothes and bedding brought with him were returned at once on the same stretcher to the ward from which he came. On a special table covered with a white rubber sheet all body hairs were clipped close below the nit-bearing level and this clipping was supplemented by shaving about the pubes and anus where body lice as well as pubic lice were often found concealed in the hair. The sheet was then removed with all hair carefully folded in it, and the hair washed into a pail and boiled. The patient was then bathed thoroughly with soap and water with the exception of the head. After drying, the head, axillae, and the pubic and anal regions were treated with a mixture of kerosene and "lightwood oil," [1] equal parts, which was also rubbed more lightly over the remainder of the body. The heads of patients were clipped and treated with oil without

[1] "Lightwood oil" is a light tar oil produced from wood by the Department of Propellants, British Ministry of Munitions, during the war. It was recovered from the top of crude pyroligneous liquors produced in the distillation of wood. It is not an ordinary product of commerce. Its efficiency according to Mr. Bacot is probably due to the presence of creosote, a low surface tension, and possibly an acid reaction. Mr. Bacot has obtained favorable results from experiments in which he tested the effect of "lightwood tar oils" obtained in commerce upon pediculi. They were cheap and efficient. They were used pure or, usually, mixed with other oils.

washing since we found reason to suspect that wetting the deep layers of dandruff on the scalps of some interfered with the penetration of the oil and allowed lice to survive. The head was finally tied up in a cloth cap to retain the oil, the patient was given clean pajamas or nightshirt and carried on a wheel-stretcher to his bed.

This lousing process was performed either by typhus-immune orderlies or by nurses in full protective dress, in each case under the supervision of physicians. All who worked in the wards wore special louse-proof gowns with closed stocking-footed trousers closely sewed beneath the skirt of the gown about the waist (Fig. 13). After each day's wear all gowns were deloused by placing them in galvanized iron cans with a generous sprinkling of crude flake naphthalene over each gown to stand undisturbed until the following day. This method was proved to be efficacious by repeatedly placing control boxes of lice among the gowns; when the gowns were removed the lice in the boxes were always dead.

In delousing, bathing, and bedmaking or in other work involving special possibilities of exposure rubber gloves fitting closely over the wrists of the gown were worn, and the wrists and neck-opening of the gown were sprayed with cedarwood oil as a repellent. The nurses wore cloth head-coverings to confine the hair. All who worked in the wards were warned to be on the lookout for lice and to conduct themselves as if lice were known to be present. It was also found necessary to delouse the Polish ward-maids and orderlies, and to inspect washing returned from outside laundries.

As precautions against transmission of complicating diseases from one patient to another cups, dishes, mouth-cleaning instruments, linen, and excretions were treated as in typhoid. In bedmaking and general care of patients the usual practice of American hospitals was followed as a practical demonstration of American methods.

The diet, by agreement with the hospital management, was that supplied in all other parts of the hospital, to which the American Red Cross contributed food in order to relieve the

FIG. 13. — Protective gowns used in the typhus wards. The nurse on the right is wearing the stocking feet of the gown outside the shoes

existing shortage. Water was given freely, and liquids and soft solids were provided according to appetite and digestion. In some unconscious patients water and liquid nourishment were given by esophageal tube. Mouths were swabbed at least five times a day with boric acid and glycerin solution, and in case of marked dryness petrolatum or boric acid ointment was applied.

Cool water sponge baths were given for temperatures of 103° or over as taken for the four-hourly chart, and in our opinion were of distinct value as in typhoid. Enemas were given every second day as needed for constipation, since although there is no theoretical objection to cathartics by mouth in typhus fever, practically they seemed to have little effect. Watch was kept for necessity for catheterization.

Digitalis or strychnia was given in most cases for possible supportive effect, and in collapse camphor or caffein, none with any manifest advantage. We were unable to use strophanthin as recommended by Danielopolu (1919, p. 327). For milder delirium or insomnia veronal or chloral were prescribed freely, especially as the Division was visited each evening, and for severe and exhausting delirium hyoscine hypodermically proved of service where the condition demanded it. Codein was much depended on to diminish tiresome cough, and contrary to some teachings we found morphia hypodermically to be of distinct service in delaying exhaustion from the frequent combination of cough, restlessness, insomnia, delirium, and general discomfort. Gangrene over points of pressure was combated by efforts to distribute pressure by pads; in one case threatened gangrene over a large area was aborted. Parotitis was treated by icebags, but all cases except one later required surgical incision. Other complications not peculiar to typhus were treated as is usual in other diseases. Some of the more important clinical observations made upon these 181 cases are presented in tabular form, Table III.

TABLE III. TABULATION OF CLINICAL DATA

Number of cases: *181*
    Men.. 86
    Women..................................... 95

Ages:

			deaths	
10-20	....	40	deaths	0
20-30	....	44	"	2
30-40	....	38	"	4
40-50	....	31	"	7
50-60	....	20	"	6
60-70	....	6	"	4
70 plus	....	2	"	1

Prodromes (non-prostrating symptoms prior to true invasion):

None.....................	21	Three days.................	24
One day..................	63	Over three days (vague)	31
Two days.................	41		

Symptoms prominent in onset:

Headache.................	161	Constipation..............	157
Chills, repeated............	84	Insomnia..................	88
Chills, single..............	50	Retention of urine..........	8
Pain, back and limb........	97	Febrile prostration, without	
Vomiting.................	53	other symptoms..........	5
Anorexia..................	77	Labial herpes..............	2

Day of disease on admission to hospital:

2d.......................	1	9th.......................	27
3d.......................	1	10th......................	14
4th......................	11	11th......................	9
5th......................	12	12th......................	3
6th......................	34	13th......................	0
7th......................	37	14th......................	1
8th......................	31		

Objective findings noted on admission:

Excitement................	45	Eruption (discrete pink, red to	
Mental dullness............	25	purple macules) present on:	
Facies tense...............	49	Trunk...................	180
Facies lax................	23	Extremities.............	142
Face flushed...............	73	Neck....................	101
Conjunctival injection		Face (few lesions)........	6
moderate........... 83		Palms (few lesions).......	2
marked............. 68		Lungs:	
extreme............ 6		Râles, musical throughout.	55
	— 157	Râles, crepitant at bases...	73
Tongue coated, moist.......	86	Cough without râles......	55
coated, somewhat dry	68	Spleen palpable............	48
very dry...............	26	Absolutely irregular heart...	8
Throat somewhat reddened..	59		

Symptoms prominent in progress:

Delirium, marked	86	Diarrhoea	17
Cough troublesome	69	Incontinence of urine	13
Vomiting	9		

Objective features in progress:

Rash markedly hemorrhagic	52	Convulsion, terminal	2
Rash overlain by mottled erythema	23	Coma	11
		Otitis media	4
Rash, some lesions palpable	14	Parotitis	7
Lungs, moist râles at bases	144	Submaxilliary adenitis	3
Lungs, signs of consolidation, not terminal	16	Gangrene	6
		Iliac thrombosis	1
Terminal bronchopneumonic symptoms with or without demonstrable consolidation	18	Deafness, marked	5
		Erysipelas, terminal	1
Trismus, marked	7	Phlegmon	3

Defervescence occurred on:

9th day	1	16th day	14
10th "	5	17th "	10
11th "	12	18th "	3
12th "	13	19th "	2
13th "	25	20th "	1
14th "	42	21st "	1
15th "	27	22d "	1

Deaths occurred on:

7th day	1	15th day	2
8th "	2	16th "	1
9th "	none	17th "	1
10th "	1	18th "	1
11th "	5	21st "	1
12th "	3	24th "	1
13th "	1	27th "	1
14th "	3		

THE WEIL–FELIX REACTION

THE Weil-Felix reactions and the necessary controls were done by Mr. Henry Pinkerton. The two cultures of *Bacillus proteus X 19* used were given to us by the Pasteur Institute in Paris, through the kindness of Dr. H. Violle. These cultures were designated as Metz and Syrie, and were respectively isolated in Metz in 1918 during the German occupation from a case of typhus from the Rhine provinces and in Syria in 1917 in a Turkish laboratory from a case of typhus from the neighborhood of Constantinople.

The technic employed was based upon the directions issued by the Department of Pathology of the University of Oxford on behalf of the Medical Research Committee. Both the macroscopic and microscopic methods were employed, carried out as follows:

1. MACROSCOPIC METHOD

Appropriate dilutions of the suspected serum were made in small agglutination tubes, by means of a graduated pipette, and an amount of the saline suspension of the organism equal to one and one-half times the volume of the diluted serum was added to each tube. The saline suspension of the organism was made by washing off the growth from a 24-hour glucose agar slant with 6 c.c of normal saline solution.

For routine work on typhus sera, the dilutions 1–100, 1–200, 1–400, 1–800, and 1–1600 were used for the Weil-Felix reaction, and dilutions of 1–25 and 1–200 were used for the Widal test. (In cases of especial interest, other dilutions were used.)

In the case of the Weil-Felix reaction, these tubes were incubated at 37° C. for two hours and the results were read after standing at room temperature for fifteen or twenty minutes. In the case of the Widal test, incubation was carried on for two

hours at 52°C. The end-point was taken as the highest dilution at which distinct clumping could be seen by holding the tube against a dark background.

MICROSCOPIC METHOD

Dilutions were made as for the macroscopic method. One loopful of the diluted serum was mixed with one loopful of the suspension of the organism on a cover-slip which was inverted over a hollow slide and incubated for fifteen minutes at 37° C.

From time to time control agglutination tests were done with sera from normal individuals, some of whom had received preventive inoculations with vaccines of *B. typhosus*, *B. paratyphosus*, A and B (indicated T, A, B in tables). Control tests were also done with the Metz and Syrie *Proteus* cultures, *B. typhosus*, *B. paratyphosus*, A and B, using stock immune sera for *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B. The results of these controls are shown in Tables IV to VI on pp. 35 and 36.

In addition to the controls tabulated, controls were done with each series of Weil-Felix tests. One tube containing the saline suspension of each culture *plus* normal salt solution and one tube containing normal human serum (No. 1 in the tables) diluted 1–50 were set up and incubated. These controls were negative in each instance.

2. TABULATION OF CONTROLS (Done by macroscopic method)

TABLE IV.   TESTS DONE ON NORMAL SERA BEFORE
BEGINNING WORK

No.	Date	T* A* B* Vaccine	End Point of Agglutination				
			T*	A*	B*	Metz*	Syrie*
1	April 5	1 yr. ago	1–400	1–800	1–400	1–10	1–10
2	" 5	10 mos. "	1–800	1–800	1–400	1–10	1–10
3	" 6	2 yrs. "	1–100	1–100	1–200	1–10	(neg. at 1–10)
4	" 6	none	1– 25	1– 25	....	1–25	1–10
5	" 6	none	1– 25	(neg. at 1–25)		(neg. at 1–10)	

For footnote, see p. 36.

TABLE V. TESTS DONE ON IMMUNE SERA

Date	End Point of Agglutination				
	T	A	B	Metz	Syrie
Test done on Typhoid immune serum					
April 14	(beyond 1–6400)	1–800	1–800	1–10	1–25
Test done on Paratyphoid A immune serum					
April 14	1–400	(beyond 1–6400)	1–800	1–10	1–25
Test done on Paratyphoid B immune serum					
April 14	1–400	1–800	(beyond 1–6400)	1–25	1–25

TABLE VI. REPETITIONS OF TESTS ON NORMAL SERA

No.	Date	TAB Vaccine	End Point of Agglutination				
			T	A	B	Metz	Syrie
1	April 17	1 yr. ago	1– 400	1– 800	1– 800	1–10	1–10
2	" 17	10 mos. "	1– 800	1– 800	1– 400	1–10	1–10
3	" 17	2 yrs. "	1– 200	1– 100	1– 200	(neg. at 1–10)	
1	" 29	1 yr. "	1– 400	1– 800	1– 800	1–10	1–10
2	" 29	10 mos. "	1– 800	1– 800	1– 400	1–10	(neg. at 1–10)
1	May 9	1 yr. "	1– 400	1– 800	1– 400	1–10	(neg. at 1–10)
2	" 9	19 days "	1–3200	1–3200	1–1600	1–50	1–50
1	" 19	1 yr. "	1– 800	1– 800	1– 400	1–10	1–10
2	" 19	29 days "	1–3200	1–6400	1–3200	1–50	1–50

* T indicates *B. typhosus*.
  A indicates *B. paratyphosus A*.
  B indicates *B. paratyphosus B*.
  Metz and Syrie refer to the two strains of *B. proteus X 19* used.

Summary of the Tables of Controls

The two *Bacillus proteus* cultures, Metz and Syrie, were very rarely agglutinated by any of the control sera, and then only in a very low dilution, the highest being 1–50. The cultures of *Bacillus typhosus*, *Bacillus paratyphosus A*, and *Bacillus para-*

typhosus B were agglutinated in very high dilution by their respective specific sera. The sera of the three inoculated persons agglutinated these cultures at about the dilutions to be expected in view of the dates of their inoculations with the T, A, B vaccine. The sera of two uninoculated individuals did not agglutinate these cultures in a higher dilution than 1-25.

3. Tabulation of Results of Agglutination Tests on Typhus Cases with Reference to Day of Illness

TABLE VII

Case No.	Day of Fever	End Point of Agglutination					
		T	A	B	Mets	Syrie	Ave-Mets and Syrie
116	4th	800	0	0	800	1600	1200
53*	5th	0	0	0	800	1600	1200
37	6th	0	0	0	....	....	....
40	"	0	0	0	50	125	88
73	"	1600	0	0	800	800	800
81	"	50	0	0	200	200	200
82	"	6400	0	0	100	100	100
83	"	0	0	0	100	100	100
117	"	400	0	0	1600	1600	1600
125	"	0	0	0	0	0	0
39	7th	0	0	0	....	....	....
51†	"	0	0	0	1600	1600	1600
78	"	50	0	0	200	200	200
32	8th	0	0	0	....	....	....
44	"	0	0	0	....	250	250
50*	"	0	0	0	3200	800	2000
54*	"	0	0	0	800	800	800
75	"	800	0	0	100	100	100
33	9th	0	0	0	....	....	....
34	"	0	0	0	....	....	....
36	"	0	0	0	....	....	....
38	"	0	0	0	....	....	....
46†	"	0	0	0	400	400	400
95	"	0	0	0	400	1600	1000
110	"	50	0	0	800	1600	1200

For footnotes, see p. 39.

TABLE VII. — Continued

Case No.	Day of Fever	End Point of Agglutination					
		T	A	B	Metz	Syrie	Ave-Metz and Syrie
112	9th	25	0	0	400	800	600
115	"	100	0	0	800	800	800
43	10th	250	0	0	50	250	150
45*	"	0	0	0	400	800	600
56	"	0	0	0	800	800	800
58	"	0	0	0	400	800	600
59	"	0	0	0	400	200	300
62	"	50	0	0	3200	800	2000
71	"	0	0	0	400	800	600
72	"	0	0	0	200	200	200
77	"	0	0	0	1600	1600	1600
84	"	0	0	0	800	800	800
85	"	0	0	0	100	100	100
94	"	100	0	0	1600	1600	1600
101	"	50	0	0	400	400	400
113	"	0	0	0	400	800	600
35	11th	0	0	0	50	250	150
80	"	50	0	0	800	800	800
96	"	50	0	0	800	1600	1200
97	"	0	0	0	800	800	800
108	"	50	0	0	400	800	600
111	"	200	0	0	800	1600	1200
114	"	25	0	0	800	1600	1200
130	"	0	0	0	0	0	0
47*	12th	0	0	0	800	800	800
57	"	50	0	0	1600	1600	1600
60	"	0	0	0	1600	1600	1600
63	"	0	0	0	1600	1600	1600
64	"	0	0	0	1600	1600	1600
69	"	0	0	0	100	100	100
79	"	800	0	0	800	3200	2000
103	"	100	0	0	200	200	200
104	"	50	0	0	800	800	800
106	"	25	0	0	800	800	800
107	"	25	0	0	400	800	600
70	13th	0	0	0	800	800	800
74	"	25	25	0	400	400	400
90	"	800	0	0	400	800	600

TABLE VII. — Continued

Case No.	Day of Fever	End Point of Agglutination					
		T	A	B	Mets	Syrie	Ave-Mets and Syrie
91	13th	50	0	0	200	800	500
92	"	50	0	0	200	400	300
98	"	0	0	0	1600	1600	1600
118	"	800	0	0	200	100	150
134	"	1600	200	400	400	800	600
48*	14th	0	0	0	1600	1600	1600
68	"	0	0	0	200	200	200
88	"	0	0	0	200	200	200
89	"	6400	50	25	400	800	600
93	"	50	0	0	800	800	800
105	"	200	0	0	800	1600	1200
133	"	1600	200	400	400	800	600
55	16th	0	0	0	400	1600	1000
131	"	0	0	0	400	800	600
122	17th	100	0	0	200	200	200
132	"	0	0	0	400	800	600
99	18th	0	0	0	3200	3200	3200
100	"	100	0	0	400	800	600
124	"	0	0	0	800	800	800
126	"	0	0	0	800	800	800
127	"	3200	0	0	0	0	0
51	19th	0	0	0	1600	1600	1600
120	21st	0	0	0	1600	1600	1600
123	"	0	0	0	200	200	200
128	"	0	0	0	400	800	600
129	"	0	0	0	1600	1600	1600
119	24th	200	0	0	800	400	600
121	"	0	0	0	800	1600	1200

* Done by microscopic method.

† Done by microscopic and macroscopic methods.

All others done by macroscopic method only.

T indicates B. typhosus.

A indicates B. paratyphosus A.

B indicates B. paratyphosus B.

Mets indicates B. proteus X 19, Mets, culture.

Syrie indicates B. proteus X 19, Syrie, culture.

Summary of the Table of Agglutination Tests on Typhus Patients

Of the eighty-three sera from typhus patients tested, only 3 or 3.6 per cent failed to agglutinate one of the *B. proteus* cultures at a dilution of 1–100. Fifty-six or 67 per cent were positive in a dilution of 1–800 or over, 26 or 31 per cent were still positive at 1–1600. The highest dilution at which agglutination was obtained was 1–3200.

The highest dilution at which agglutination was obtained with any of the eight non-typhus sera was 1–50.

Our results are in accord with those of many other workers who have tested the value of the Weil-Felix reaction. The test is valuable as a supplement to clinical observation in the diagnosis of typhus. It should be employed as part of the routine work of the laboratory of every hospital receiving typhus cases.

V

LOUSE FEEDING EXPERIMENTS UPON TYPHUS PATIENTS

EXPERIMENTS DONE TO ASCERTAIN THE NATURE AND INCIDENCE OF MICRO-ORGANISMS ACQUIRED BY LICE FROM TYPHUS CASES

1. METHODS OF FEEDING

THE lice used were enclosed in boxes as described in the section on "Technic." Thirty to forty lice, usually larvae and nymphs, were placed in each box. The immature stages were used to avoid the too great increase in numbers and consequent danger in handling the boxes, which would result if adults were employed. Also, if adults were used some would die from old age before the termination of the three weeks usually allowed for feeding; while their progeny probably would have fed for too short a time to permit the development of a heavy infection in them of any organism derived from the patient.

Fifty-two experiments were carried to completion in a series of sixty-five. In the first six experiments the louse boxes were left continuously attached to the patients for the whole period of feeding, six to nine days. This method was discarded for a number of reasons: it caused discomfort to the patients, it prevented close supervision of the boxes containing lice, and it gave the lice but one opportunity to feed during the early days of a typhus infection when the blood is said to be most infective (Nicolle, 1920), (von Prowazek, 1915–16), (da Rocha-Lima, 1919–21).

In all experiments, after the sixth, the lice were fed twice daily, morning and evening; the boxes being left in position for one-half hour to an hour at each feeding. The patients selected for the feeding experiments were all clinically well-established cases of typhus and, as far as possible, in the early stages of the disease.

As a rule the louse boxes were applied to the outer surface of the leg. This region is easily manipulated and the skin is not sensitive. The lice will not feed through the bolting cloth unless the boxes are firmly applied to the skin and kept immobilized; the death of the lice in a number of experimental boxes in our early experiments was due to starvation through failure to realize this. Another cause of the loss of lice was the placing of boxes on patients whose skins were still greasy with the mixture of oils used in delousing. The lice in one box were killed by a few drops of alcohol accidentally dropped on it.

In view of the possibility of infection by the feces from infected lice, great care was taken to avoid scattering the dry feces. Gloves were worn while applying the boxes and cloths wet with a solution of bichloride of mercury were placed upon a rubber sheet beneath the patient's leg in order to catch the droppings. The box, when applied, was covered with an ample pad of absorbent cotton and fixed in position by a firmly applied bandage. At the end of the feeding, the bandage was cut, the louse box returned to an individual receptacle (glass Stender dish), the bandage and cotton placed in a paper bag for burning, and the skin of the leg disinfected with alcohol.

Between feedings, the louse boxes, each in a separate glass dish, were kept in an incubator. The temperature of the incubator is a very important factor. Early in our experiments, when the incubator temperature was kept at 20°–25° C. positive findings in the lice were rare, while toward the end of the experiments, when the incubator was maintained in the neighborhood of 30°C. some of the lice in each box invariably were infected with rickettsia. Other factors influencing favorably the acquisition of rickettsia by lice were length of time allowed for each feeding, extended to one hour, and the perfect immobilization of the boxes; both were observed in our later experiments. The usual period covered by the feeding of a box was twelve to sixteen days, but occasionally it was extended to twenty-four or more days up to twenty-nine.

2. Results of the Louse Feeding Experiments

The results and important data of the louse feeding experiments are tabulated in Table VIII.

The answer to the first question awaiting solution — "the determination of the nature of the demonstrable micro-organisms acquired by lice nurtured upon typhus patients" was clean cut — *Rickettsia prowazeki.* The occasional finding of this micro-organism in a series of largely negative experiments in the first part of our work led to changes, mentioned above, in temperature of incubator, duration of the daily feedings and duration of the whole period of feeding; after the changes were made, lice in experimental boxes were almost always infected with this micro-organism.

In the fifty-two experiments, *Rickettsia prowazeki* appeared in the lice of twenty-seven. With the recognition of the conditions favorable for infection of the lice, we were able to secure almost uniformly positive results. Rickettsia appeared in lice in each of the last thirteen consecutive feeding experiments and in eighteen out of the last twenty-one consecutive experiments following the longer feedings and raising of the incubator temperature to 30°C. The identification of the rickettsia as *Rickettsia prowazeki* was in each instance based upon appearances seen in serial sections of lice; although the incidence of infected lice in each box was largely determined by smear preparations.

In lice from six boxes we found a few infected with an extracellular rickettsia (Figs. 32 and 64, plates IX and XXVI), which, from its distribution, deep staining and morphology we believe to be *Rickettsia pediculi.*

In some lice there was a double infection with intra and extracellular rickettsia (Fig. 64, plate XXVI). The source of this infection with *Rickettsia pediculi* can with considerable constancy be traced to three of several patients upon whom five of these boxes were fed (Boxes XLVII, L, LI, LIII, LIV, Experiments 35, 38, 39, 41, 42). The symptoms in each patient were those of typhus; nothing in the history or in the clinical course

TABLE VIII. LOUSE FEEDING EXPERIMENTS

Number of Experiment	Number of Louse Box	Dates of Feeding From	To	Stock of Lice Used	Temperature of Incubator	Numbers of Patients Used	Day of Disease of Patient Used	Smears R−	Smears R+	Sections R−	Sections R ped.+	Sections R prow.+
1	I	Apr. 3–Apr.	9	W	25°+C	18	12–18	8		7		
2	III	" 13 "	30	T	"	33	12–16	2		2		
						82	4– 8					
3	IV	" 13 "	24	T	"	73	12–20	4		11		
						38	4– 7					
4	V	" 14 "	26	W	"	40	9–17	6		6		
						70	12–14					
5	VI	" 14 "	24	W	"	44	12–17	5		9		2
						73	4– 7					
6	VII	" 14 "	26	W	"	37	11–23	8		5		
7	VIII	" 14 "	30	W	"	46	3–10	5		5		
						53	5–15					
						49	10					
						80	9–13					
8	IX	" 16–May	3	B	"	51	9–19	1	7	3		3
						66	11–15					
						76	14–18					
9	X	" 16 "	3	B	"	49	7–18	10		8		
						53	15–21					
						68	8					
10	XI	" 16 "	4	B	"	52	7–18	8		7		
						76	14–21					
11	XII	" 16 "	3	B	"	56	5–16	5	3	4		3
						72	13–15					
						68	17–21					
12	XIII	" 18 "	3	W	"	63	9–14	4	4	3		4
						69	15					
13	XIV	" 18 "	10	W	"	62	7–15	8		5		
						83	4–18					
						80	17					
14	XV	" 18 "	10	W	"	50	10–11	6		6		
						57	10–15					
						69	11–19					
						89	11–21					
15	XVI	" 18 "	3	W	"	61	7–19	4		7		
						90	10–14					
16	XVII	" 18 "	15	W	25°–30°	60	10–32	4	2	2		3
						113	9–14					
17	XVIII	" 23 "	15	W	"	75	4– 9	4	2	2		1
						86	7–15					
						106	8–14					
						121	10–13					

B, T, and W indicate lice nurtured upon Baoot, Todd, and Wolbach respectively.

TABLE VIII — Continued

Number of Experiment	Number of Louse Box	Dates of Feeding — From	Dates of Feeding — To	Stock of Lice Used	Temperature of Incubator	Numbers of Patients Used	Day of Disease of Patient Used	Lice Examined — Smears R−	Smears R+	Sections R−	Sections R ped. +	Sections R prow. +
18	XIX	Apr. 22–	May 15	W	25°–30°	73	5–13	5	1	6		1
						63						
						93	11–17					
						102	4– 9					
						115	9–13					
19	XXI	" 23	" 16	W	"	74	12–20	4		3		
						63						
						87	7–11					
						99	11–13					
						83	17					
						82	10–13					
						111	10–14					
						113	13					
20	XXII	" 24	" 16	W	"	78	3–14	7				
						99	11–16					
						103	12–18					
21	XXIII	" 27	" 18	T	"	81	5–19	8	1	5		1
						104	11–18					
22	XXIV	" 27	" 15	T	"		2– 5	8		6		
						42	29–44					
23	XXVII	" 28	" 18	W	"	82	6–19	11		4		
						111	10–12					
						115	10–14					
						127	10–12					
24	XXVIII	" 28	" 19	W	"	80	11–14	4		5		
						82	9–18					
						111	10–13					
						113	12–18					
25	XXXIV	May 7–	" 22	W	"	107	9–14	4		2		
						121	10–17					
						132	9–12					
26	XXXVI	" 7	" 24	W	"	110	8–17	1		1		
						121	14–19					
						132	12–14					
						125	14					
27	XXXVII	" 10	" 24	W	"	100	18–25	12		4		
						127	11					
						125	9–11					
						131	9–14					
28	XXXVIII	" 10	" 24	W	"	116	4–15	6	7	6		
						134	8					
29	XL	" 10	" 25	W	"	118	12–17	4		4		
						122	7–13					
						136	11–15					
30	XLI	" 16	" 26	W	30°	122	8–12	3		6		
						136	10–15					

TABLE VIII — Continued

Number of Experiment	Number of Louse Box	Dates of Feeding From	To	Stock of Lice Used	Temperature of Incubator	Numbers of Patients Used	Day of Disease of Patient Used	Smears R−	Smears R+	Sections R−	R ped. +	R prow. +
31	XLII	May 16–May 28		W	30°	103	17–18			6		
						129	12–16					
						139	8–10					
						140	7– 9					
						134	10–12					
						147	10					
						155	7					
32	XLIII	" 16–June 7		W	30°	115	14–17	7	2	3		1
						104	20					
						136	10					
						130	7–14					
						148	10					
						155	7–11					
						162	6					
						167	7–12					
33	XLIV	" 16–May 26		W	30°	126	10–14	16		6		
						123	15					
						140	6–11					
34	XLV	" 16–June 1		W	30°	123	11–16			4		
						140	6–12					
						150	9					
35	XLVII	" 16 " 13		W	30°	127	10–17		6	1	1	3
						132	14					
						144	8					
						145	7					
						148	9–10					
						157	8–12					
						160	8					
						161	8–10					
						170	8–14					
						175	11					
						179	4					
36	XLVIII	" 16 " 12		W	30° 32°	117	14–15	4	4	2		3
						128	12–16					
						133	7–13					
						148	10					
						155	7–11					
						162	6					
						167	7–15					
38	L	" 19 " 2		T	30° 32°	130	9–17	1	7	3	1	4
						133	5–13					
						134	8–16					
						135	6–14					
39	LI	" 19 " 1		T	30° 32°	133	8–16	8	1	3	1	1
						134	6–14					
						137	8–16					

TABLE VIII — Continued

Number of Experiment	Number of Louse Box	Dates of feeding		Stock of Lice Used	Temperature of Incubator	Numbers of Patients Used	Day of Disease of Patient Used	Lice Examined				
								Smears		Sections		
		From	To					R −	R +	R −	R ped. +	R prow. +
40	LII	May 19–June 2		T	30° 32°	130 134 135 137	8–16 6–14 8–16 8–16	9		12		
41	LIII	" 19	" 2	T	30° 32°	130 133 134 137	7–10 7–15 9–17 7–15	8			2	2
42	LIV	" 22	" 15	W	30° 32°	139 143 148 157	9–12 10 8–11 8–11		12		1	3
43	LV	" 22	" 15	W	30° 32°	160 161 170 175 141 147 149 158 168 172 180	8 8–11 8–14 11 9–12 7 7–11 5– 8 7–16 13 5	6	6	2		2
44	LVI	" 22	" 15	W	30° 32°	131 136 143 144 152 161 162 178 177 182	12–14 14 10 8–10 7–11 7 7–16 9 12–14 12	1	2			3
45	LVII	" 22	" 17	W	30° 32°	127 132 144 145 152 162 167 177	16 14 8–10 7 7–12 6 7–16 11	1	11			6
46	LVIII	" 22	" 12	W	30° 32°	142 146 153 163 172	6– 9 8–10 7–12 7–11 8–11	4	7			4

TABLE VIII — Continued

Number of Experiment	Number of Louse Box	Dates of Feeding From	To	Stock of Lice Used	Temperature of Incubator	Numbers of Patients Used	Day of Disease of Patient Used	Lice Examined Smears R −	R +	Sections R −	R ped. +	R prow. +
47	LIX	May 22–June 12		W	30°	142	6– 9	3	8	1		3
					32°	146	8–10					
						153	7–12					
						159	10					
						163	7–11					
						172	8–12					
48	LX	" 22	" 17	W	30°	141	9–12	5	3	1		3
					32°	147	7					
						149	7–11					
						158	5– 8					
						168	7–12					
						172	13					
						180	5–10					
49	LXI	" 25	" 15	W	32°	147	8–10	9	2	4		
						151	9–14					
						161	7					
						162	7–16					
						178	9–13					
50	LXII	" 25	" 15	W	32°	147	8–10	10	1	3		1
						151	9–13					
						161	7					
						162	7–16					
						178	9–13					
51	LXIII	" 25	" 15	W	32°	145	8–11	8	1	4		
						156	6–12					
						163	8–11					
						172	8–13					
						177	11					
						182	12					
						168	13					
52	LXIV	" 25	" 17	W	32°	145	8–11	11	3	3		1
						156	6–13					
						161	10					
						170	8–14					
						175	11					
						179	9–11					
						180	7					
53	LXV	" 25	" 17	W	32°	140	11–12	11	5	5	1	2
						150	8–12					
						158	5– 8					
						168	7–13					
						172	10–14					
						180	5–10					

of any case, during the time it was under our observation, gives clinical ground for suspecting the existence of trench fever. None of the cases were related nor came from a common address. It is of interest to note that in Box LII, Experiment 40, though fed only upon patients used in feeding the doubly infected lice, neither *Rickettsia prowazeki* nor *Rickettsia pediculi* appeared.

The occasional occurrence of *Rickettsia pediculi* was anticipated by us because of our previous experience with the lice from the Warsaw bath-house from which Mr. Bacot presumably became infected with trench fever.

Micro-organisms other than rickettsia did not appear in any of the fifty-two experiments as the result of the feeding upon typhus patients. The cocco-bacillus, found in the genital tract of one louse from Box VI, Experiment 5, was familiar to us from our initial controls of the English stock lice. The lice of Box VI, however, were of the American stock and this was the first instance of the occurrence of this bacterium in the American stock.

We have found it to be more difficult to infect lice with *Rickettsia prowazeki* than is apparently indicated in the accounts of da Rocha-Lima (1916, p. 29). Even in heavily infected boxes in the later half of our work there was always a varying percentage of lice in which rickettsia could not be demonstrated, and in Box LII not one of the twenty-one lice recovered and examined, twelve by serial sections, nine by smears, showed rickettsia. It is certain that these negative lice were exposed equally with their companions to rickettsia infection. No suggestion can be advanced by us in explanation for the absence of rickettsia in these lice; but, as shown below, we have proved that only a similar proportion of the lice fed upon typhus patients acquire the virus of typhus. In Box LII a few lice showed appearances in cells of the alimentary tract which later may be proved to be indications of early infection with *Rickettsia prowazeki*.

In these experiments no attempt was made to ascertain the infectivity of patients at various stages of the disease as shown

by the appearance of rickettsia in lice fed upon them at different days of the disease. The prime object of these experiments was to ascertain what organism was constantly found in lice nurtured upon typhus patients. So our louse boxes were placed as often as possible upon patients in the first days of the fever; the work of previous observers (Nicolle, 1920), (Prowazeki, 1915–16), (da Rocha-Lima, 1919–21) had indicated that the blood was most infectious during the first period of the disease. Consequently, the boxes in the latter half of our work were fed upon at least four different patients and frequently upon eight patients.

VI

EXPERIMENTS TO PROVE THE SPECIFICITY OF *RICKETTSIA PROWAZEKI* FOR TYPHUS

THESE experiments were undertaken originally with two objects in view. The failure to infect all the lice with *Rickettsia prowazeki* in boxes in which some became infected; in spite of the fact that all had equal opportunities to become infected to the extent that they were equally well nurtured upon the same patients made it necessary to ascertain if all or only a number of the lice in such boxes acquired the virus of typhus. This was the first object of these experiments. The second object was to ascertain if the presence of rickettsia was essential for the infectivity of the louse.

1. METHODS

Owing to the difficulty of feeding lice upon guinea-pigs, it was decided to test their infectivity by injecting intraperitoneally into guinea-pigs emulsions of the viscera of the lice. It was previously shown by Nicolle (1911, 1912), Ricketts and Wilder (1910), da Rocha-Lima (1916[1]) and others that monkeys and guinea-pigs could be infected with typhus by this method; da Rocha-Lima (1916[1]) maintained with greater certainty than by the injection of blood from typhus patients. Accordingly, as time and guinea-pigs permitted, guinea-pigs were injected with lice from a number of boxes selected at random before examination for rickettsia.

The lice were dissected with sterile instruments upon sterile slides and the organs teased apart and suspended in sterile salt solution. A sterile syringe was used for the injection of the material from each louse. A portion of the material used for inoculation was spread upon a slide and stained with Giemsa's stain for subsequent search for rickettsia.

Forty-two guinea-pigs were inoculated (each with one louse) from ten different boxes. The temperature of each guinea-pig

was taken daily by a uniform technic in order to give an indication of the probable results of the inoculations. At the time these experiments were begun we were seriously handicapped for lack of a sufficient number of normal guinea-pigs. Our stocks of monkeys and guinea-pigs were suffering from paratyphoid infections which in the guinea-pigs in the chronic stage took the form of a pseudo-tuberculosis. We were forced to use many guinea-pigs which had passed through this infection but which had apparently recovered as evidenced by a period of a week or more of normal temperature. In several instances this infection was relighted as an effect of the typhus infection. It apparently shortened the incubation period of typhus and prolonged the febrile period. The final conclusions as to the results were based upon one or more of the following controls: histological examination of the brain and other organs for lesions characteristic of typhus; subinoculation of other guinea-pigs; reinoculation with blood of typhus patients as a test of immunity. The human bloods used in testing the immunity of the guinea-pigs at the conclusions of the experiments were proved to be infective for fresh normal guinea-pigs. Lice from Boxes XVII, XVIII, XIX, XXI, XXII, XXVIII, XXXIV, XXXVI, XXXVII, and XXXVIII were used for these experiments (see Table VIII of the 52 louse feeding experiments). Owing to the importance of these experiments they are given in detail. A summary of the results is presented in Table IX, p. 110.

2. Louse Feeding Specificity Experiments

Experiments with Box XVII lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 25°C. The box contained 30 W lice (6 adult males, 4 adult females, 20 nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
April 18............	....	6.30	Placed on Case 60	10th
" 19............	11.00	5.30	" " "	11th
" 20............	12.00	6.00	" " "	12th
" 21............	12.00	5.00	" " "	13th
" 22............	11.00	6.00	" " "	14th
" 23............	11.30	6.00	" " "	15th
" 24............	12.30	6.15	" " "	16th
" 25............	12.15	....	" " "	17th
" 26............	11.30	6.30	" " "	18th
" 27............	12.30	5.45	" " "	19th
" 28............	12.30	6.30	" " "	20th
" 29............	12.15	....	" " "	21st
" 30............	12.00	....	" " "	22d
May 1............	12.00	6.30	" " "	23d
" 2............	12.30	6.30	" " "	24th
" 3............	12.30	6.45	" " "	25th
" 4............	12.30	....	" " "	26th
" 5............	12.45	....	" " "	27th
" 6............	11.30	....	" " "	28th
" 7............	10.00	6.30	" " "	29th
" 8............	10.00	6.30	" " "	30th
" 9............	11.00	9.00	" " "	31st
" 10............	12.30	....	Transferred to Case 113	9th
" 11............	12.30	6.15	" " "	10th
" 12............	10.15	....	" " "	11th
" 13............	11.00	....	" " "	....
" 15............	10.00		Box removed; kept at 20° to 25° C. until box opened; it contained feces, eggs, 1 well-fed and 1 dead nymph; no larvae, 12 living and 14 dead adult lice.	

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears		
W 236	Rickettsia absent	Guinea-pig 11; Temp. — Proved susceptible to blood from a typhus patient
W 237	Rickettsia absent	Guinea-pig 17; Temp. — Proved susceptible to blood from a typhus patient
W 238	Rickettsia present	Guinea-pig 20; Temp. + Lesions present. Blood proved infectious in subinoculations
W 239	Rickettsia absent	Guinea-pig 19; Temp. + Lesions present. Blood proved infectious in subinoculations
W 240	Rickettsia absent	Guinea-pig 21; Temp. —
W 241	Rickettsia present	Guinea-pig 22; Temp. +
Sections		
W 228		
W 230	Rickettsia present	
W 231	Rickettsia present	
W 232[1]	Rickettsia present	
W 232[2]	Rickettsia absent	

Record of Guinea-pig 11, inoculated with rickettsia-free louse W 236

From May 15th to June 22d the temperature of this guinea-pig remained within normal limits. On June 22d it was tested for susceptibility by the injection of 5 c.c. of blood from patient 799. The temperatures subsequent to this inoculation were as follows:

	°F.			°F.
June 22	No record		July 6	No record
" 23	103.3		" 7	105.4
" 24	No record		" 8	104.4
" 25	101.8		" 9	No record
" 26	No record		" 10	102.0
" 27	102.6		" 11	103.1
" 28	No record		" 12	102.1
" 29	102.8		" 13	102.4
" 30	102.4		" 14	101.9
July 1	104.2		" 15	No record
" 2	No record		" 16	101.6
" 3	104.4		" 17	102.5
" 4	105.4		" 18	Killed
" 5	105.3			

The spleen was enlarged. A caseous lymph node of healed pseudo-tuberculosis was found. No other lesions.

Result: Guinea-pig 11 failed to react, after inoculation with the viscera of louse W 236, during an observation period of thirty-eight days. It then proved susceptible to an injection of blood from a typhus patient.

Conclusion: Louse W 236 did not contain the virus of typhus.

Record of Guinea-pig 17, inoculated with rickettsia-free louse W 237

From May 15th to June 22d the temperature of this guinea-pig remained normal. On June 22d it received intraperitoneally 5 c.c. of blood from patient 799. The temperatures subsequent to this inoculation were as follows:

	°F.			°F.
June 22	No record	July	6	No record
" 23	103.2	"	7	104.4
" 24	No record	"	8	105.0
" 25	102.0	"	9	No record
" 26	No record	"	10	103.4
" 27	102.0	"	11	102.1
" 28	No record	"	12	102.6
" 29	103.1	"	13	101.8
" 30	102.1	"	14	102.8
July 1	103.4	"	15	No record
" 2	No record		16	102.2
" 3	104.7	"	17	102.6
" 4	106.8	"	18	Killed
" 5	106.0			

No gross lesions were found.

Result: Guinea-pig 17 failed to react, after inoculation with the viscera of louse W 237, during an observation period of thirty-eight days. It proved susceptible to an injection of blood from a typhus patient.

Conclusion: Louse W 237 did not contain the virus of typhus.

Record of Guinea-pig 20, inoculated with rickettsia-infected louse W 238

The temperatures following the inoculation on May 15th were as follows:

	°F.			°F.
May 15	102.4		May 24	103.5
" 16	102.2		" 25	105.0
" 17	102.0		" 26	104.7
" 18	102.0		" 27	103.8
" 19	102.4		" 28	103.4
" 20	102.4		" 29	105.0
" 21	101.5		" 30	104.1
" 22	101.8		" 31	104.2
" 23	102.0			

May 31st; killed. The autopsy was completely negative except for a slightly enlarged spleen.

The histological examination showed the lesions characteristic of typhus in the brain. Two other guinea-pigs, Nos. 69 and 70, inoculated intraperitoneally with 2 c.c. of blood from guinea-pig 20, remained with normal temperatures until June 14th when each developed temperature and ran a course typical of typhus in the guinea-pig, of seven days duration in the case of No. 69 and five days duration with No. 70. Following this their temperatures remained normal for a further observation period of nineteen and twenty-one days respectively.

Result: Guinea-pig 20, inoculated with rickettsia-infected louse W 238, developed typhus after an incubation period of nine to ten days. The presence of typhus was proved by histological examination and by subinoculations of two other guinea-pigs.

Conclusion: Rickettsia-infected louse W 238 contained the virus of typhus.

*Record of Guinea-pig 19, inoculated with rickettsia-infected
louse W 239*

The temperatures following the inoculation on May 15th
were as follows:

	°F.			°F.
May 15	101.5	May 21		105.0
" 16	101.9	" 22		105.0
" 17	102.7	" 23		104.6
" 18	102.7	" 24		105.5
" 19	106.3	" 25		104.4
" 20	105.2			

May 25th; killed. The spleen was slightly enlarged and
covered with a thin transparent fibrin-like layer. In the liver
were two hard white nodules, evidently of healed pseudo-
tuberculosis. The other tissues were negative. Cultures of
blood from the heart proved sterile three days later.

The histological examination showed the characteristic brain
lesions of typhus (see p. 194).

Three other guinea-pigs, Nos. 50, 51, and 52, were inoculated
intraperitoneally each with 2 c.c. of blood from the heart of
guinea-pig 19.

Guinea-pig 50 developed temperature on June 7th which
lasted five days, following which it was normal and on June
28th it was tested for immunity by the intraperitoneal injec-
tion of 5 c.c. of blood from patient 814 (eighth day of typhus).
During a further observation period of twenty days its tem-
perature remained normal.

Guinea-pig 51 developed temperature on May 6th which
lasted for seventeen days. From June 25th to June 28th tem-
perature was normal. On June 28th it was inoculated intra-
peritoneally with 5 c.c. of blood from patient 814 (eighth day of
typhus). During a further observation period of twenty days
its temperature remained normal.

Guinea-pig 52 developed no characteristic temperature re-
action but between June 2d and 10th had occasional elevations
to 104°F. On May 30th it was tested by an intraperitoneal in-
oculation with 5 c.c. of blood from patient 828 (tenth day of

typhus) and during a further observation period of eighteen days its temperature remained normal.

Result: Guinea-pig 19, inoculated with rickettsia-infected louse W 239, developed typhus apparently after an incubation period of four days. The presence of typhus was proved by histological examination and by subinoculations and immunity tests upon three other guinea-pigs.

Conclusion: Rickettsia-infected louse W 239 contained the virus of typhus.

Record of Guinea-pig 21, inoculated May 15th with rickettsia-free louse W 240

Guinea-pig 21's temperature did not exceed normal limits between May 15th and June 22d, a period of thirty-eight days. It died June 26, following an inoculation of blood on June 22d as an immunity test. The autopsy was negative. There was no histological control.

Result: Guinea-pig 21 did not develop a period of temperature consistent with typhus, and probably did not have typhus.

Conclusion: Rickettsia-free louse W 240 probably did not contain the virus of typhus.

Record of Guinea-pig 22, inoculated May 15th with rickettsia-infected louse W 241

Guinea-pig 22's temperature rose on May 19th and remained high (103.5°F. to 105°F.), with occasional remissions of one to several days until June 15th, when it remained normal until June 27th, when it rose to 104°F. On June 22d it had received 5 c.c. of blood as an immunity test. It died July 1st and the autopsy showed active pseudo-tuberculosis.

Result: Guinea-pig 22 probably developed typhus, though the course was modified by a pseudo-tuberculosis infection which was probably previously latent.

Conclusion: Louse W 241 probably contained the virus of typhus.

Experiments with Box XVIII lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 25°C. The box contained 30 W lice (2 adult males, 3 adult females, 25 nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
April 23............	12.00	6.00	Placed on Case 75	4th
" 24............	12.30	6.45	" " "	5th
" 25............	12.00	....	" " "	6th
" 26............	11.45	6.45	" " "	7th
" 27............	11.00	6.00	" " "	8th
" 28............	12.45	6.45	Transferred to Case 86	7th
" 29............	12.30	6.30	" " "	8th
" 30............	12.00	....	" " "	9th
May 1............	12.00	6.30	" " "	10th
" 2............	12.30	6.30	" " "	11th
" 3............	12.30	6.15	" " "	12th
" 4............	12.30	....	" " "	13th
" 5............	11.00	....	" " "	14th
" 6............	11.30	....	" " 106	8th
" 7............	10.15	6.30	" " "	9th
" 8............	10.15	6.00	" " "	10th
" 9............	12.15	9.00	" " "	11th
" 10............	12.45	....	" " "	12th
" 11............	12.15	6.30	" " "	13th
" 12............	10.30	....	" " 121	10th
" 13............	11.15	.. ..	" " "	11th
" 15............	11.30		Box removed, kept at 20° to 25° C. until box opened; it contained much feces, many eggs, 1 dead larva, a few dead nymphs, 14 dead and 11 living lice.	

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears		
W 223	Rickettsia absent	Guinea-pig 24; Temp. + No control
W 224	Rickettsia present	Guinea-pig 23; Temp. + Lesions present. Controlled by positive subinoculations
W 225	Rickettsia absent	Guinea-pig 16; Temp. − Proved susceptible to blood from a typhus patient
W 226	Rickettsia absent	Guinea-pig 10; Temp. − Proved susceptible to blood from a typhus patient
W 227	Rickettsia present	Guinea-pig 9; Temp. + Proved immune to blood from a typhus patient
W 228	Rickettsia present	Guinea-pig 15; Temp. + Lesions present
Sections		
W 217	Rickettsia absent	
W 218	Rickettsia absent	
W 219	Rickettsia present	

Record of Guinea-pig 24, inoculated with the viscera of rickettsia-free louse W 223

The temperatures of guinea-pig 24 were as follows:

	°F.		°F.
May 15	102.4	May 23	104.2
" 16	102.3	" 24	104.2
" 17	102.6	" 25	104.3
" 18	102.4	" 26	104.6
" 19	102.9	" 27	104.4
" 20	105.2	" 28	102.4
" 21	104.0	" 29	Dead
" 22	104.6		

The autopsy showed pneumonia and pseudo-tuberculosis.

Result: It is very probable that this guinea-pig did receive the virus of typhus and that the course was influenced by the previously existing though latent pseudo-tuberculosis infection.

Conclusion: Rickettsia-free louse W 223 possibly contained the virus of typhus. An inconclusive experiment.

Record of Guinea-pig 23, inoculated with rickettsia-infected louse W 224

The temperatures following the inoculation on May 15th were as follows:

	°F.			°F.
May 15	102.9	May 22		102.4
" 16	103.0	" 23		102.6
" 17	102.6	" 24		102.6
" 18	102.7	" 25		102.8
" 19	103.0	" 26		105.0
" 20	102.7	" 27		105.0
" 21	103.1	" 28		105.2

May 28th; killed. The autopsy was negative except for an enlarged spleen covered with a tissue paper thin layer of transparent fibrin-like material. Histologically the characteristic lesions of typhus were found in the brain.

Three guinea-pigs, Nos. 58, 59, and 60, were inoculated intraperitoneally, each with 2.5 c.c. of blood from this guinea-pig, No. 23.

The temperatures of these guinea-pigs were as follows:

	Guinea-pig 58	Guinea-pig 59	Guinea-pig 60
	°F.	°F.	°F.
May 29	103.8	102.4	103.6
" 30	103.4	102.7	103.0
" 31	102.1	101.4	102.9
June 1	101.8	101.4	102.0
" 2	102.0	102.2	102.9
" 3	101.0	101.0	102.0
" 4	102.2	102.6	102.8
" 5	102.2	102.0	102.0
" 6	103.7	103.6	104.0
" 7	105.0	104.5	105.0
" 8	105.4	105.6	105.0
" 9	105.0	105.3	105.0

These animals were killed June 9th. The autopsies of all were consistent with typhus in guinea-pigs. Guinea-pig 60 had nodules of pseudo-tuberculosis in spleen, liver, and omentum, but the lungs were normal.

Histological examination showed the lesions characteristic of typhus in the brains of all three guinea-pigs. From guinea-pig 59, guinea-pigs 78 and 79 were inoculated intraperitioneally each with 2.5 c.c. of blood.

From guinea-pig 60, guinea-pigs 76 and 77 were inoculated intraperitoneally each with 2.5 c.c. of blood.

The temperatures of these four guinea-pigs were as follows:

	Guinea-pig 76	Guinea-pig 77	Guinea-pig 78	Guinea-pig 79
	°F.	°F.	°F.	°F.
June 10	103.6	102.6	103.8	102.4
" 11	103.6	102.4	104.0	102.0
" 12	103.8	102.2	104.0	102.4
" 13	103.4	102.6	103.8	102.3
" 14	102.8	103.0	103.6	102.2
" 15	102.6	102.8	104.0	101.4
" 16	....	....	....	....
" 17	....	....	....	....
" 18	104.5	104.8	104.4	102.4
" 19	105.7	104.1	103.8	101.6
" 20	....	....	....	....
" 21	106.1	104.6	102.6	104.0
" 22	....	....	....	....
" 23	105.8	104.6	104.3	104.0
" 24	105.2	104.2	104.8	104.6
" 25	104.6	103.2	103.8	104.6 killed
" 26	....	....	....	....
" 27	103.0	102.0	....	....
" 28	....	....	105.0	....
" 29	103.2	102.4	....	....
" 30	103.1	102.6	103.4	....
July 1	102.6	102.4	103.3	....
" 2	....	....	102.6	....
" 3	104.2	104.4	104.2	....
" 4	102.6	102.5	102.2	....
" 5	102.4	....	101.6	....
" 6	....	102.3	....	....
" 7	102.2	....	101.5	....
" 8	102.1	101.9	102.7	....
" 9	....	101.8	....	....
" 10	102.2	101.6	102.1	....

The autopsy on guinea-pig 79 showed a much enlarged spleen covered with a thin transparent fibrin-like layer. All other tissues were negative. From this guinea-pig the strain

has been maintained up to the present time, and on several occasions guinea-pigs of this louse Box XVIII strain have been studied histologically, and have shown the characteristic lesions of typhus in the brains.

Result: Guinea-pig 23, inoculated with rickettsia-infected louse W 224, developed typhus after an incubation period of eleven days. The infection was proved by histological examination and subinoculations, the strain having been carried up to the present time.

Conclusion: Rickettsia-infected louse W 224 contained the virus of typhus.

Record of Guinea-pig 16, inoculated with viscera from rickettsia-free louse W 225

The temperatures following inoculation on May 15th remained normal. On June 22d, after an observation period of thirty-eight days, this guinea-pig 16 was inoculated intraperitoneally with 5 c.c. of blood from patient 799 (eighth day of typhus).

The subsequent temperatures were as follows:

		°F.				°F.
June	22	No record	July	5		105.8
"	23	103.1	"	6		No record
"	24	No record	"	7		105.3
"	25	102.2	"	8		104.9
"	26	No record	"	9		No record
"	27	102.4	"	10		103.6
"	28	No record	"	11		104.1
"	29	102.2	"	12		102.9
"	30	102.4	"	13		102.6
July	1	104.3	"	14		102.4
"	2	No record	"	15		No record
"	3	103.9	"	16		102.4
"	4	104.9	"	17		102.1

July 19th; killed. The autopsy was wholly negative except for fibrous adhesions in the abdominal cavity.

Result: Rickettsia-free louse W 225 did not transmit typhus to guinea-pig 16, during an observation period of thirty-eight days. When inoculated with blood from a typhus patient, this

guinea-pig developed a temperature consistent with typhus infection, after a period of ten days. This probably indicates a susceptibility of this guinea-pig to typhus.

Conclusion: Rickettsia-free louse W 225 probably did not contain the virus of typhus.

Record of Guinea-pig 10, inoculated with viscera from rickettsia-free louse W 226

The temperatures following inoculation on May 15th showed rises on May 18, 19, 24, and 25, and June 18, to 103.5°F. or higher, but upon intervening days was within normal limits. On June 22, after an observation period of thirty-eight days, the guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 799 and the temperatures subsequently were as follows:

		°F.				°F.
June	22	No record	July	5		105.7
"	23	103.6	"	6		No record
"	24	No record	"	7		No record
"	25	102.3	"	8		104.9
"	26	No record	"	9		No record
"	27	102.2	"	10		104.1
"	28	102.3	"	11		104.2
"	29	No record	"	12		103.8
"	30	102.4	"	13		102.0
July	1	103.2	"	14		102.1
"	2	No record	"	15		No record
"	3	104.3	"	16		102.1
"	4	105.4	"	17		101.6

June 17th; killed. The autopsy showed a slightly enlarged spleen. Other tissues were negative except for cicatrices in liver and omentum.

Result: Rickettsia-free louse W 226 did not transmit typhus to guinea-pig 10 during an observation period of thirty-eight days. After inoculation with blood from a typhus patient this guinea-pig developed typhus, after a further observation period of eleven or twelve days, thereby proving the susceptibility of this guinea-pig.

Conclusion: Rickettsia-free louse W 226 did not contain the virus of typhus.

Record of Guinea-pig 9, inoculated with the viscera of
rickettsia-free louse W 227

The temperatures subsequent to inoculation on May 15th
were as follows:

	°F.		°F.
May 15	102 4	May 23	104.4
" 16	102.1	" 24	104.5
" 17	No record	" 25	103.9
" 18	103.0	" 26	103.8
" 19	105.4	" 27	104.0
" 20	104.4	" 28	103.0
" 21	104.4	" 29	102.0
" 22	104.8		

From May 29th to June 22d the temperatures remained
within normal limits. On June 22d this guinea-pig 9 received
intraperitoneally 5 c.c. of blood from typhus patient 799
(eighth day of disease). No rise of temperature followed (ex-
cept on July 3d when it rose to 104.5°F. in common with that
of all other guinea-pigs on the first day of transit in an over-
heated freight car) during a further observation period of
twenty-five days.

Result: Guinea-pig 9, inoculated with viscera from rickettsia-
infected louse W 227 developed typhus after an incubation
period of four or five days. It proved later to be immune to
blood from a typhus patient which was infectious for other
guinea-pigs. An autopsy on July 18th gave completely nega-
tive results. No histological examination was made.

Conclusion: Rickettsia-infected louse W 227 contained the
virus of typhus.

*Record of Guinea-pig 15, inoculated with the viscera of
rickettsia-infected louse W 228*

The temperatures following inoculation on May 15th were as
follows:

	°F.			°F.
May 15	102.4	May 22		104.0
" 16	102.6	" 23		103.1
" 17	102.1	" 24		104.4
" 18	102.4	" 25		104.4
" 19	103.0	" 26		104.8
" 20	102.7	" 27		105.2
" 21	103.1	" 28		105.0

May 28th; killed. The spleen was markedly enlarged and
covered with a thin transparent fibrin-like layer. All other tis-
sues were negative.

The brain lesions characteristic of typhus were found upon
histological examination.

Result: Guinea-pig 15 developed typhus after inoculation
with viscera from louse W 228 after an incubation period of
eight to ten days. The infection was proved by histological
examination.

Conclusion: Rickettsia-infected louse W 228 contained the
virus of typhus.

Experiments with Box XIX lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 25°C. The box contained 30 W lice (2 adult males, 3 adult females, 25 nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.			Day of Disease
	A.M.	P.M.				
April 22............	....	6.00	Placed on Case 73			5th
" 23............	11.30	6.00	"	"	"	6th
" 24............	12.30	6.30	"	"	"	7th
" 25............	12.00	....	"	"	"	8th
" 26............	11.30	6.45	"	"	"	9th
" 27............	12.45	6.00	"	"	"	10th
" 28............	12.30	6.45	"	"	"	11th
" 29............	12.30	....	"	"	"	12th
" 30............	12.00	....	Transferred to Case 63			13th 21st
May 1............	12.00	....	"	"	93	11th
" 2............	12.30	6.30	"	"	"	12th
" 3............	12.30	6.45	"	"	"	13th
" 4............	12.30	....	"	"	"	14th
" 5............	12.30	....	"	"	"	15th
" 6............	....	1.30	"	"	"	16th
" 7............	10.15	6.30	"	"	102	4th
" 8............	10.15	6.00	"	"	"	5th
" 9............	12.15	9.00	"	"	"	6th
" 10............	12.45	....	"	"	"	....
" 11............	12.15	6.00	"	"	115	9th
" 12............	10.00	....	"	"	"	10th
" 13............	10.45	....	"	"	"	11th
" 14............	11.30	....	"	"	112	12th
" 15............	....		Box removed, kept at 20° to 25° C. until box opened; it contained feces, eggs, 1 young well-fed and few old dead larvae, 1 well-fed small nymph, 15 living and 7 dead adult lice.			

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears		
W 211	Rickettsia present	Guinea-pig 13; Temp. +
		Proved immune to inoculation
W 212	Rickettsia absent	Guinea-pig 7; Temp. −
		Proved susceptible to inoculation
W 213	Rickettsia absent	Guinea-pig 14; Temp. +
		Lesions present
W 214	Rickettsia absent	Guinea-pig 8; Temp. +
		Proved immune to inoculation
W 215	Rickettsia absent	Guinea-pig 18; Temp. +
		Lesions present
W 216	Rickettsia absent	Guinea-pig 12; Temp. −
		Proved susceptible to inoculation with blood from a typhus patient
Sections		
W 203	Rickettsia absent	
W 204 [1]	Rickettsia absent	
W 204 [2]	Rickettsia present	
W 205	Rickettsia absent	
W 206 [1]	Rickettsia absent	
W 206 [2]	Rickettsia absent	
W 208	Rickettsia absent	

*Record of Guinea-pig 13, inoculated with the viscera of
rickettsia-infected louse W 211*

The temperatures following the inoculation on May 15th
were as follows:

		°F.				°F.
May	15	101.7	June	3		104.6
"	16	102.2	"	4		103.0
"	17	102.4	"	5		104.0
"	18	102.9	"	6		104.0
"	19	102.3	"	7		103.4
"	20	102.6	"	8		103.0
"	21	103.3	"	9		102.0
"	22	102.2	"	10		103.0
"	23	104.0	"	11		103.0
"	24	102.5	"	12		102.4
"	25	103.2	"	13		102.4
"	26	102.8	"	14		102.8
"	27	103.0	"	15		102.0
"	28	103.2	"	16		103.2
"	29	103.6	"	17		No record
"	30	104.2	"	18		103.6
"	31	104.0	"	19		103.2
June	1	104.2	"	20		No record
"	2	104.4	"	21		105.0

On June 22d this guinea-pig 13 was inoculated intraperi-
toneally with 5 c.c. of blood from patient 799 (eighteenth day of
typhus). Its temperatures subsequently were as follows:

	°F.			°F.
June 23	104.0	July 10		102.0
" 24	No record	" 11		102.2
" 25	102.9	" 12		102.1
" 26	No record	" 13		101.8
" 27	103.0	" 14		101.9
" 28	103.1	" 15		No record
" 29	No record	" 16		102.1
" 30	103.3	" 17		101.7
July 1	104.0	" 18		102.0
" 2	No record	" 19		101.8
" 3	104.0	" 20		102.0
" 4	102.8	" 21		No record
" 5	103.0	" 22		102.5
" 6	No record	" 23		102.6
" 7	102.1	" 24		103.0
" 8	102.1	" 25		101.7
" 9	No record			

Result: Guinea-pig 13, inoculated with rickettsia-infected
louse W 211 developed a reaction consistent with typhus fifteen
or sixteen days later. Following an injection of 5 c.c. of typhus
blood of known infectivity it failed to develop temperatures
consistent with typhus during an observation period of thirty-
three days, thereby proving immunity to typhus.

Conclusion: Rickettsia-infected louse W 211 contained the
virus of typhus.

Record of Guinea-pig 7, inoculated with the viscera of
rickettsia-free louse W 212

The temperature, following the inoculation on May 15th,
rose on one day, May 23, to 104°F. At no other time did the
temperature pass out of the normal range until June 21st when
it was 103.5°F. On June 22d, after this observation period of
thirty-eight days, it received intraperitoneally 5 c.c. of blood
from patient 799 (eighth day of typhus) with the following
result:

		°F.			°F.
June	22	No record	July	5	106.0
"	23	103.8	"	6	No record
"	24	No record	"	7	105.4
"	25	No record	"	8	105.0
"	26	No record	"	9	No record
"	27	102.4	"	10	103.2
"	28	No record	"	11	102.7
"	29	102.4	"	12	102.8
"	30	102.4	"	13	103.1
July	1	103.0	"	14	102.2
"	2	No record	"	15	No record
"	3	104.5	"	16	102.1
"	4	105.8	"	17	102.2

July 17th; killed. The autopsy showed a slightly enlarged spleen. There were no lesions in other tissues.

Result: Guinea-pig 7, inoculated with louse W 212, during an observation period of thirty-eight days failed to develop temperatures suggestive of typhus. Following an inoculation of typhus blood, after a further observation period of ten or eleven days, it developed temperatures and a course typical of typhus.

Conclusion: Rickettsia-free louse W 212 did not contain the virus of typhus.

Record of Guinea-pig 14, inoculated with the viscera of rickettsia-free louse W 213

The temperatures following the inoculation on May 15th were as follows:

		°F.			°F.
May	15	102.3	May	22	104.2
"	16	102.0	"	23	104.0
"	17	101.9	"	24	104.6
"	18	102.4	"	25	104.6
"	19	102.6	"	26	104.3
"	20	102.2	"	27	104.2
"	21	103.4	"	28	104.0

May 28th; killed. The autopsy showed an enlarged spleen covered with a thin transparent fibrin-like layer. No lesions were found in any other tissues.

On histological examination the lesions characteristic of typhus were found in the brain.

Result: Guinea-pig 14, inoculated with louse W 213, apparently free of rickettsia, developed a course typical of typhus after an incubation period of eight days. The infection was proved to be typhus by histological examination.

Conclusion: Louse W 213, a preparation of which contained no rickettsia, contained the virus of typhus.

Record of Guinea-pig 8, inoculated with the viscera of rickettsia-free louse W 214

	°F.			°F.
May 15	102.1	May 27		104.2
" 16	101.4	" 28		104.0
" 17	104.0	" 29		103.8
" 18	104.4	" 30		103.6
" 19	104.4	" 31		104.0
" 20	104.5	June 1		103.8
" 21	105.0	" 2		103.6
" 22	104.6	" 3		102.6
" 23	104.4	" 4		103.9
" 24	104.3	" 5		103.2
" 25	104.0	" 6		103.0
" 26	102.9	" 7		102.4

From June 7th to June 21st the temperatures were normal. On June 22d this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 799 (eighth day of typhus); the following temperatures resulted:

	°F.			°F.
June 23	103.4	July 6		No record
" 24	No record	" 7		102.4
" 25	No record	" 8		102.0
" 26	No record	" 9		No record
" 27	102.6	" 10		103.0
" 28	No record	" 11		102.4
" 29	102.4	" 12		102.6
" 30	102.1	" 13		102.7
July 1	103.3	" 14		103.2
" 2	No record	" 15		No record
" 3	103.8	" 16		102.2
" 4	104.2	" 17		102.0
" 5	104.2			

Discussion: The rise of temperature in guinea-pig 8, after the inoculation with louse W 214, was too early for typhus, but not inconsistent with the reaction in a guinea-pig harboring a mild or latent infection. The duration of the temperature covered

the usual period of a typhus infection and incubation period, so that we must accept the temperatures of guinea-pig 8 as rather indicative of typhus than otherwise. The short temperature rise on July 3, 4, and 5 coincided with very hot weather in transport in a freight car when many guinea-pigs showed elevated temperatures.

Result: Guinea-pig 8, inoculated with louse W 214, apparently free of rickettsia, developed typhus fever. That the infection was typhus was shown by the failure to react typically following inoculation with human blood of proved infectivity. (The brain of this guinea-pig, and those of others killed upon the same date, were lost. Hence there is no histological control.)

Conclusion: Louse W 214, a preparation of which contained no rickettsia, probably contained the virus of typhus.

Record of Guinea-pig 18, inoculated with the viscera of rickettsia-free louse (nymph) W 215

The temperatures following the inoculation on May 15th were as follows:

	°F.		°F.
May 15	101.9	May 24	102.3
" 16	102.6	" 25	102.9
" 17	102.9	" 26	104.3
" 18	102.4	" 27	103.8
" 19	102.8	" 28	104.0
" 20	102.8	" 29	104.0
" 21	103.6	" 30	104.1
" 22	102.7	" 31	104.0
" 23	101.8		

May 31st; killed. The autopsy showed an enlarged spleen covered with a thin transparent fibrin-like layer. All other tissues were negative.

On histological examination the lesions characteristic of typhus were found.

Result: Guinea-pig 18, inoculated with the viscera of louse W 215, a preparation of which contained no rickettsia, developed typhus, which was proved by histological examination.

Conclusion: Louse W 215 (nymph), in a preparation of which no rickettsia were found, contained the virus of typhus.

Record of Guinea-pig 12, inoculated with rickettsia-free
louse W 216

At no time during an observation period of thirty-eight days did this guinea-pig's temperature exceed normal limits. On June 22d it was inoculated intraperitoneally with 5 c.c. of blood from patient 799 (eighth day of typhus) as a test for immunity. The temperatures subsequent to this inoculation were:

	°F.			°F.
June 22	No record	July	5	106.8
" 23	103.4	"	6	No record
" 24	No record	"	7	103.8
" 25	102.2	"	8	103.8
" 26	No record	"	9	No record
" 27	102.0	"	10	103.1
" 28	102.1	"	11	102.4
" 29	No record	"	12	102.8
" 30	102.2	"	13	102.7
July 1	104.0	"	14	102.4
" 2	No record	"	15	No record
" 3	103.8	"	16	102.1
" 4	105.9	"	17	102.6

July 17th; killed. The spleen was slightly enlarged; no other lesions found.

Result: Guinea-pig 12, inoculated with the viscera of rickettsia-free louse W 216, did not develop typhus in an incubation period of thirty-eight days. It proved susceptible to an inoculation of human blood on June 22d, developing typhus after a further observation period of nine days.

Conclusion: Rickettsia-free louse W 216 did not contain the virus of typhus.

Experiments with Box XXI lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 25°C. The box contained 30 W lice (2 adult males, 3 adult females, 25 nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
April 23.............	11.30	5.30	Placed on Case 74	12th
" 24.............	12.15	6.30	" " "	13th
" 25.............	12.00	....	" " "	14th
" 26.............	11.45	5.45	" " "	15th
" 27.............	12.45	6.00	" " "	16th
" 28.............	12.30	6.45	" " "	17th
" 29.............	12.30	6.45	" " "	18th
" 30.............	12.00	....	Transferred to Case 63	21st
May 1.............	12.00	6.30	" " 87	7th
" 2.............	12.15	6.30	" " "	8th
" 3.............	12.30	6.30	" " "	9th
" 4.............	12.30	....	" " "	....
" 5.............	....	1.00	" " 99	11th
" 6.............	....	1.30	" " "	12th
" 7.............	10.15	6.30	" " 83	17th
" 8.............	10.30	....	" " 82	16th
	....	5.45	" " "	16th
" 9.............	12.15	9.00	" " "	17th
" 10.............	12.30	....	" " 111	10th
" 11.............	12.45	6.00	" " "	11th
" 12.............	10.00	....	" " "	12th
" 13.............	10.45	....	" " "	13th
" 14.............	11.30	....	" " 113	13th
" 16.............	10.45		Box removed, kept at 20° to 30° C. usually 29° until box opened; it contained feces, many eggs, a few larvae, a few dead old nymphs, 7 living and 6 dead adult lice.	

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears		
W 261	Rickettsia absent	Guinea-pig 25. Died in five days
W 262	Rickettsia absent	Guinea-pig 26; Temp. + Histology negative
W 263	Rickettsia absent	Guinea-pig 27; Temp. −
W 264	Rickettsia absent	Guinea-pig 28; Temp. − Proved susceptible to blood from a typhus patient
Sections		
W 258	Rickettsia absent	
W 259	Rickettsia absent	
W 260	Rickettsia absent	

Record of Guinea-pig 25, inoculated with the viscera of rickettsia-free louse W 261

This guinea-pig died on the fifth day, of pneumonia.

Record of Guinea-pig 26, inoculated with the viscera of rickettsia-free louse W 262

The temperatures following the inoculation on May 16th were as follows:

	°F.			°F.
May 16	101.8		May 27	102.0
" 17	102.4		" 28	102.0
" 18	102.6		" 29	101.8
" 19	102.4		" 30	102.1
" 20	102.1		" 31	103.0
" 21	102.0		June 1	105.0
" 22	102.2		" 2	105.0
" 23	102.4		" 3	105.0
" 24	No record		" 4	105.4
" 25	101.9		" 5	104.8
" 26	101.3			

June 5th; killed. Cultures from the heart's blood in dextrose broth remained sterile (fifty-six hours). The autopsy was negative except for an enlarged spleen.

Microscopic examination was negative for all tissues, including the brain.

Three guinea-pigs, Nos. 71, 72, and 73, were inoculated intraperitoneally each with 3 c.c. of blood from the heart of guinea-pig 26.

Guinea-pigs 71 and 73 developed high temperatures, 105.8°F. and 106°F. on the ninth and eighth days respectively. No. 71 died June 14th and autopsy showed peritonitis and active pseudo-tuberculosis. No. 73 was killed on June 20, up to which time the temperature remained high. Autopsy showed fibrinous peritonitis and active pseudo-tuberculosis. The histological examination of the brain was completely negative.

Guinea-pig 72 survived the inoculation and ran the following course of temperature:

		°F.			°F.
June	6	102.8	June	18	102.6
"	7	103.0	"	19	102.6
"	8	103.6	"	20	No record
"	9	102.3	"	21	102.4
"	10	102.4	"	22	No record
"	11	103.0	"	23	102.4
"	12	103.2	"	24	102.2
"	13	102.8	"	25	102.6
"	14	104.0	"	26	No record
"	15	102.4	"	27	No record
"	16	No record	"	28	102.6
"	17	No record			

On June 28th, preparatory to our departure from Warsaw, this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 814 (eighth day of typhus).

The subsequent temperatures were as follows:

		°F.			°F.
June	28	102.6	July	8	102.0
"	29	No record	"	9	No record
"	30	102.4	"	10	102.6
July	1	102.2	"	11	102.7
"	2	No record	"	12	102.8
"	3	103.8	"	13	102.0
"	4	104.5	"	14	103.0
"	5	104.0	"	15	No record
"	6	No record	"	16	102.9
"	7	102.4	"	17	102.8

There is no record of the fate of this guinea-pig. The observations were concluded preparatory to departure from Paris.

Result: Guinea-pig 26, inoculated with the viscera of ricket-tsia-free louse W 262, after an incubation period of sixteen days, developed temperatures consistent with typhus. Although it was not killed until the fifth day of temperature, no lesions were found in the brain. One of three guinea-pigs, No. 72, which survived inoculation from this guinea-pig, failed to develop typhus during an observation period of twenty-two days, at the end of which time it was inoculated with blood from a typhus patient. No temperature suggestive of typhus developed in a further observation period of nineteen days.

Discussion: Guinea-pig 26 developed a course of fever consistent with typhus, though after an incubation period of unusual length, following louse injections. The failure to find lesions in the brain of this guinea-pig, and that of No. 73 inoculated from it, is strong evidence against typhus. In no instance in a series of thirty-six consecutive brains examined of guinea-pigs infected with typhus with blood, human and guinea-pig, were the brain lesions absent and we are inclined to accept the absence of brain lesions in guinea-pigs 26 and 73 as proof of the absence of typhus. The failure of guinea-pig 72 to develop typhus following inoculation from No. 26 is offset by its failure to react to the inoculation with the blood of patient 814.

Conclusion: Rickettsia-free louse W 262 probably did not contain the virus of typhus.

*Record of Guinea-pig 27, inoculated with the viscera of
rickettsia-free louse W 263*

The temperatures following inoculation on May 16th were as
follows:

	°F.			°F.
May 16	102.1	May 27		105.0
" 17	102.8	" 29		104.2
" 18	102.9	" 30		104.2
" 19	102.8	" 31		102.4
" 20	102.8	June 1		102.2
" 21	102.9	" 2		104.0
" 22	102.8	" 3		103.1
" 23	103.0	" 4		103.0
" 24	No record	" 5		103.6
" 25	102.9	" 6		102.4
" 26	103.1	" 7		Dead

The autopsy showed acute enteritis, acute peritonitis and
pleuritis. No histological examination was made.

Result: The reaction of this guinea-pig is inconsistent with
typhus with or without a complicating infection.

Conclusion: Rickettsia-free louse W 263 (probably?) did not
contain the virus of typhus.

*Record of Guinea-pig 28, inoculated with the viscera of
rickettsia-free louse W 264*

The temperatures following the inoculation on May 16th
were as follows:

	°F.			°F.
May 16	102.0	May 29		104.0
" 17	102.2	" 30		103.9
" 18	101.9	" 31		103.6
" 19	102.6	June 1		104.4
" 20	103.4	" 2		102.8
" 21	103.0	" 3		102.2
" 22	103.5	" 4		103.6
" 23	103.5	" 5		103.0
" 24	103.1	" 6		103.2
" 25	103.0	" 7		102.4
" 26	103.0	" 8		103.0
" 27	103.8	" 9		103.4
" 28	104.0	" 10		102.8

	°F.			°F.
June 11	105.0		June 17	No record
" 12	103.6		" 18	104.0
" 13	103.8		" 19	103.6
" 14	104.6		" 20	No record
" 15	103.2		" 21	103.6
" 16	104.2			

On June 22d this guinea-pig was inoculated intraperitoneally with 3 c.c. of blood from patient 799 (eighth day of disease). Subsequent temperatures were as follows:

	°F.			°F.
June 22	No record		July 4	105.4
" 23	104.0		" 5	105.2
" 24	No record		" 6	No record
" 25	No record		" 7	104.7
" 26	No record		" 8	105.2
" 27	104.0		" 9	No record
" 28	104.1		" 10	103.8
" 29	No record		" 11	103.4
" 30	103.3		" 12	102.7
July 1	104.7		" 13	102.4
" 2	No record		" 14	102.3
" 3	105.0		" 15	Dead

Result: During an observation period of thirty-seven days following the inoculation with louse W 264, guinea-pig 28 ran an irregular temperature, not typical and perhaps inconsistent with typhus. Following inoculation with typhus blood it behaved in a manner typical of guinea-pigs with typhus complicated with a secondary infection, thereby demonstrating lack of immunity.

Conclusion: Rickettsia-free louse W 264 did not contain the virus of typhus.

Experiments with Box XXII lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 25°C. The box contained 30 W lice (larvae and first moult nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
April 24.............	12.30	6.15	Placed on Case 78	3d
" 25.............	12.00	....	" " "	4th
" 26.............	11.45	6.30	" " "	5th
" 27.............	12.45	6.00	" " "	6th
" 28.............	12.30	6.30	" " "	7th
" 29.............	12.30	6.30	" " "	8th
" 30.............	12.00	....	" " "	9th
May 1.............	12.00	6.30	" " "	10th
" 2.............	12.30	6.30	" " "	11th
" 3.............	12.30	6.45	" " "	12th
" 4.............	12.30	....	" " "	13th
" 5.............	....	1.00	Transferred to Case 99	11th
" 6.............	....	1.30	" " "	12th
" 7.............	10.15	6.30	" " "	13th
" 8.............	10.15	7.00	" " "	14th
" 9.............	12.30	9.15	" " "	15th
" 10.............	12.45	....	" " 103	12th
" 11.............	12.15	6.30	" " "	13th
" 12.............	10.30	....	" " "	14th
" 13.............	11.15	....	" " "	15th
" 14.............	11.30	....	" " "	16th
" 16.............	10.45		Box removed, kept at 20° to 30° C. usually 29° until box opened; it contained no feces, no eggs, a few dead larvae and nymphs, 3 living adult lice, one being very red, another moribund.	

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears		
W 265	Rickettsia absent	Guinea-pig 29; Temp. — Doubtful immunity test
W 266	Rickettsia absent	Guinea-pig 30; Temp. — Proved susceptible to blood from a typhus patient
W 267	Rickettsia absent	Guinea-pig 31; Temp. —

Record of Guinea-pig 29, inoculated with rickettsia-free louse W 265

The temperatures following inoculation on May 16th were as follows:

	°F.			°F.
May 16	101.4	June 4		102.4
" 17	103.9	" 5		103.0
" 18	103.0	" 6		103.2
" 19	103.2	" 7		102.8
" 20	103.4	" 8		102.4
" 21	102.8	" 9		101.8
" 22	102.2	" 10		102.2
" 23	102.8	" 11		102.6
" 24	102.8	" 12		102.0
" 25	103.0	" 13		101.8
" 26	103.2	" 14		102.0
" 27	103.4	" 15		102.0
" 28	102.6	" 16		No record
" 29	101.4	" 17		No record
" 30	102.0	" 18		102.0
" 31	102.2	" 19		102.4
June 1	103.0	" 20		No record
" 2	103.0	" 21		102.4
" 3	102.2			

On June 22d this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 799 (eighth day of typhus). The temperatures were as follows:

	°F.			°F.
June 22	No record	July 1		102.5
" 23	104.2	" 2		No record
" 24	No record	" 3		104.2
" 25	103.6	" 4		103.6
" 26	No record	" 5		103.8
" 27	102.8	" 6		No record
" 28	102.5	" 7		102.6
" 29	No record	" 8		102.7
" 30	102.7	" 9		No record

		°F.			°F.
July	10	.... 102.0	July	18	.... 102.0
"	11	.... 102.0	"	19	.... 101.7
"	12	.... 102.1	"	20	.... 102.1
"	13	.... 102.6	"	21	.... No record
"	14	.... 102.0	"	22	.... 102.5
"	15	.... No record	"	23	.... 102.0
"	16	.... 102.2	"	24	.... 102.0
"	17	.... 101.9	"	25	.... 102.2

Result: Guinea-pig 29 did not react to the injection of viscera of louse W 265, during an observation period of thirty-seven days. Following an injection of human typhus blood of proved infectivity it failed to react satisfactorily during a further observation period of thirty-three days.

Conclusion: The negative result of the louse inoculation carries more weight than the result of the immunity test; because, the failure of a guinea-pig to react to the inoculation of proved infective blood is common. Yet, we do not feel warranted in drawing a positive conclusion from this experiment. Nevertheless, we do conclude that the presence of the virus of typhus was not proved in louse W 265.

Record of Guinea-pig 30, inoculated with rickettsia-free louse W 266

This guinea-pig's temperature did not exceed normal limits between May 16th, the date of inoculation, and June 22d. On June 22d it was inoculated intraperitoneally with 5 c.c. of blood from patient 799 (eighth day of typhus) and the subsequent temperatures were as follows:

		°F.			°F.
June	22	.... No record	July	5	.... 102.8
"	23	.... 104.0	"	6	.... No record
"	24	.... No record	"	7	.... 103.4
"	25	.... 103.9	"	8	.... 102.6
"	26	.... No record	"	9	.... No record
"	27	.... 104.0	"	10	.... 101.8
"	28	.... 103.1	"	11	.... 103.0
"	29	.... No record	"	12	.... 102.1
"	30	.... 103.2	"	13	.... 102.8
July	1	.... 102.6	"	14	.... 101.9
"	2	.... No record	"	15	.... No record
"	3	.... 104.3	"	16	.... 104.4
"	4	.... 102.8	"	17	.... 104.1

July 17th; killed, owing to departure from Paris. The autopsy showed an enlarged spleen and no other lesions. No histological examination was made, as the tissues were lost.

Result: Guinea-pig 30, inoculated with rickettsia-free louse W 266 did not develop typhus during an observation period of thirty-seven days. Following an inoculation with human typhus blood, it apparently reacted positively after a further observation period of twenty-three days.

Conclusion: Rickettsia-free louse W 266 probably did not contain the virus of typhus.

Record of Guinea-pig 31, inoculated with the viscera of rickettsia-free louse W 267

The temperatures following inoculation on May 16th were as follows:

	°F.			°F.
May 16	102.0	May 27		101.2
" 17	102.8	" 28		101.8
" 18	102.7	" 29		102.4
" 19	102.4	" 30		102.7
" 20	102.6	" 31		104.0
" 21	102.5	June 1		104.0
" 22	102.2	" 2		102.8
" 23	102.3	" 3		102.2
" 24	102.9	" 4		102.4
" 25	102.7	" 5		103.6
" 26	102.2	" 6		Dead

The autopsy showed acute pneumonia of both lungs, acute endocarditis, and acute peritonitis.

Result: Guinea-pig 31 failed to develop typhus during the period of observation of twenty days, terminated by an acute infection.

Conclusion: Rickettsia-free louse W 267 probably did not contain the virus of typhus.

Experiments with Box XXVIII lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 25°C. The box contained 30 W lice (30 larvae).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
April 28............	12.30	6.30	Placed on Case 80	11th
" 29............	12.30	6.30	" " "	12th
" 30............	12.00	....	" " "	13th
May 1............	12.00	6.30	Transferred to Case 82	9th
" 2............	12.30	6.30	" " "	10th
" 3............	12.30	6.45	" " "	11th
" 4............	12.30	....	" " "	12th
" 5............	12.45	....	" " "	13th
" 6............	....	1.30	" " "	14th
" 7............	10.00	6.30	" " "	15th
" 8............	10.00	6.45	" " "	16th
" 9............	12.15	9.00	" " "	17th
" 10............	12.30	....	" " 111	10th
" 11............	12.45	6.00	" " "	11th
" 12............	10.00	....	" " "	12th
" 13............	10.48	....	" " 113	12th
" 14............	12.00	....	" " "	13th
" 15............	....	1.00	" " "	14th
" 16............	12.00	6.00	" " "	15th
" 17............	11.30	6.30	" " "	16th
" 18............	10.30		Box removed, kept at 20° to 30° C. usually 29° until box opened; it contained feces, eggs, no live larvae, a few dead nymphs, 13 living and several dead adult lice.	
" 19............	10.00			

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears		
I	Rickettsia absent	
II	" "	
III	" "	
IV	" "	
Smear of egg	" "	
Smear of excreta	" "	
Smear of excreta	" "	

Louse No. *Smears of lice*	Result of Microscopical Examination	Result of Animal Inoculation
1	Rickettsia absent	Guinea-pig 32; Temp. — Proved susceptible to blood of a typhus patient
2	Rickettsia absent	Guinea-pig 33. Died in ten days, of pneumonia
3	Rickettsia absent	Guinea-pig 34; Temp. — Proved susceptible to blood of a typhus patient
4	Rickettsia absent	Guinea-pig 35. Died in seven days; pseudo-tuberculosis
Sections 6	Rickettsia absent	
7	" "	
8	" "	
9	" "	
10 [1]	" "	

Record of Guinea-pig 32, inoculated with the viscera of louse 1 of Box XXVIII

The temperatures following the inoculation on May 19th were as follows:

		°F.			°F.
May	19	102.0	June	5	102.0
"	20	103.0	"	6	102.0
"	21	102.8	"	7	101.4
"	22	102.2	"	8	102.0
"	23	102.8	"	9	101.8
"	24	101.8	"	10	102.2
"	25	102.7	"	11	102.4
"	26	103.6	"	12	102.0
"	27	103.4	"	13	102.2
"	28	102.2	"	14	103.0
"	29	103.4	"	15	102.2
"	30	103.2	"	16	101.8
"	31	103.0	"	17	No record
June	1	103.8	"	18	101.8
"	2	102.8	"	19	101.9
"	3	102.2	"	20	No record
"	4	102.4	"	21	102.1

On June 22d this guinea-pig was inoculated with 5 c.c. of blood from patient 799 (eighth day of typhus) and the subsequent temperatures were as follows:

		°F.				°F.
June	22	No record	July	7		105.4
"	23	103.2	"	8		105.1
"	24	No record	"	9		No record
"	25	102.5	"	10		104.6
"	26	No record	"	11		104.8
"	27	102.6	"	12		105.1
"	28	No record	"	13		100.0
"	29	102.3	"	14		101.2
"	30	102.1	"	15		No record
July	1	102.8	"	16		104.1
"	2	No record	"	17		102.6
"	3	105.2	"	18		100.2
"	4	105.8	"	19		103.1
"	5	106.5	"	20		103.6
"	6	No record				

There is no record of an autopsy.

Result: Guinea-pig 32 did not acquire typhus, during an observation period of thirty-four days, as a result of the inoculation with louse 1 of Box xxviii. It did react in a manner typical of typhus, complicated by a previously existing infection, eleven days after the injection of typhus blood from a patient.

Conclusion: Louse 1 of Box xxviii did not contain the virus of typhus.

Record of Guinea-pig 33, inoculated with the viscera of louse 2 of Box XXVIII

The temperature of this guinea-pig rose to 104°F. the day following the inoculation, on May 19th, and remained high (104° to 105°F.) until the day preceding its death on June 29th. The autopsy showed an extensive active infection with pseudo-tuberculosis involving both chest and abdomen.

Result: Valueless.

*Record of Guinea-pig 34, inoculated with the viscera of
louse 3 of Box XXVIII*

The temperatures of this guinea-pig, following the inoculation on May 19th, were as follows:

	°F.			°F.
May 19	104.6		June 9	102.4
" 20	103.0		" 10	102.0
" 21	104.2		" 11	102.2
" 22	105.3		" 12	102.0
" 23	103.3		" 13	102.2
" 24	104.3		" 14	103.0
" 25	104.0		" 15	102.4
" 26	104.2		" 16	No record
" 27	104.4		" 17	No record
" 28	104.0		" 18	103.8
" 29	103.6		" 19	103.0
" 30	103.4		" 20	No record
" 31	102.6		" 21	102.6
June 1	103.4		" 22	No record
" 2	103.2		" 23	102.8
" 3	102.6		" 24	No record
" 4	103.0		" 25	101.8
" 5	103.0		" 26	No record
" 6	103.4		" 27	101.8
" 7	103.8		" 28	101.9
" 8	103.2			

On June 28th this guinea-pig received intraperitoneally 5 c.c. of blood from patient 814 (eighth day of typhus). The subsequent temperatures were as follows:

	°F.			°F.
June 29	No record		July 9	No record
" 30	102.0		" 10	102.4
July 1	102.0		" 11	102.3
" 2	No record		" 12	102.4
" 3	105.7		" 13	104.1
" 4	102.2		" 14	104.8
" 5	102.9		" 15	No record
" 6	No record		" 16	104.5
" 7	102.4		" 17	104.0
" 8	102.4			

July 17th; killed. The autopsy showed healed pseudo-tuberculosis of liver and spleen. The chest was normal. There was no histological examination, as the tissues were lost.

Result: The initial temperatures of this guinea-pig corresponded to those of number 33 and in the light of the autopsy were probably due to pseudo-tuberculosis. The reaction to human blood after an incubation period of thirteen days was characteristic of typhus and proof that the animal was not immune as a result of the louse injection.

Conclusion: Louse 3 of Box xxvIII did not contain the virus of typhus.

Record of *Guinea-pig 35, inoculated with the viscera of louse 4 of Box XXVIII*

The temperatures following the inoculation on May 19th were as follows:

	°F.			°F.
May 19	100.3	May 24		104.3
" 20	103.4	" 25		104.1
" 21	105.0	" 26		104.0
" 22	104.4	" 27		Dead
" 23	103.3			

The autopsy showed active pseudo-tuberculosis.

Result: Valueless. The course of this guinea-pig parallels those of numbers 33 and 34, and illustrates the behavior of the epizoötic then prevailing in our stock animals.

Experiments with Box XXXIV lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 30° C., usually 29° C. The box contained 30 W lice (30 nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
May 8............	....	6.00	Placed on Case 107	9th
" 9............	12.30	9.10	" " "	10th
" 10............	12.45	....	" " "	11th
" 11............	12.20	6.30	" " "	12th
" 12............	10.30	....	Transferred to Case 121	10th
" 13............	11.15	....	" " "	11th
" 14............	12.00	....	" " "	12th
" 15............	....	1.00	" " "	13th
" 16............	11.45	7.30	" " "	14th
" 17............	11.30	6.30	" " "	15th
" 18............	10.30	7.30	" " "	16th
" 19............	12.00	....	" " 132	9th
" 20............	11.30	7.30	" " "	10th
" 21............	11.30	....	" " "	11th
" 22............	A.M.		Box removed, kept at 20° to 30° C. usually 29° C. until box opened; it contained a little feces, a few eggs, no nymphs or larvae, 11 living lice.	

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears 1	Rickettsia absent	Guinea-pig 36; Temp. — Did not react to blood from a typhus patient
2	Rickettsia absent	Guinea-pig 37; Temp. — Proved susceptible to blood from a typhus patient
3	Rickettsia absent	Guinea-pig 38; Temp. — Proved susceptible to blood from a typhus patient
4	Rickettsia absent	Guinea-pig 39; Temp. — Experiment complicated by pseudo-tuberculosis, but proved susceptible to blood from a typhus patient
Excreta	Rickettsia absent	
Eggs	Rickettsia absent	
Sections W 11[1]	Rickettsia absent	
W 11[2]	Rickettsia absent	

*Record of Guinea-pig 36, inoculated with the viscera of
louse 1 of Box XXXIV*

Temperatures following the inoculation on May 22d:

	°F.		°F.
May 22 .... No record		June 10 .... 102.4
" 23 .... 103.0		" 11 .... 101.0
" 24 .... 102.2		" 12 .... 101.8
" 25 .... 103.1		" 13 .... 102.0
" 26 .... 102.4		" 14 .... 102.8
" 27 .... 103.2		" 15 .... 102.0
" 28 .... 103.0		" 16 .... 102.0
" 29 .... 103.4		" 17 .... No record
" 30 .... 103.1		" 18 .... 103.0
" 31 .... 102.8		" 19 .... 102.6
June 1 .... 102.0		" 20 .... No record
" 2 .... 102.2		" 21 .... 103.0
" 3 .... 101.8		" 22 .... No record
" 4 .... 101.0		" 23 .... 102.6
" 5 .... 101.8		" 24 .... No record
" 6 .... 102.0		" 25 .... 102.0
" 7 .... 101.8		" 26 .... No record
" 8 .... 102.0		" 27 .... 101.6
" 9 .... 102.2		

On June 28th this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 814. The subsequent temperatures were as follows:

	°F.		°F.
June 28 .... 102.4		July 12 .... 103.1
" 29 .... No record		" 13 .... 102.8
" 30 .... 102.2		" 14 .... 103.4
July 1 .... 102.1		" 15 .... No record
" 2 .... No record		" 16 .... 102.6
" 3 .... 104.3		" 17 .... 103.0
" 4 .... 103.3		" 18 .... 102.4
" 5 .... 103.0		" 19 .... 102.9
" 6 .... No record		" 20 .... 102.1
" 7 .... 103.2		" 21 .... No record
" 8 .... 102.9		" 22 .... 103.2
" 9 .... No record		" 23 .... No record
" 10 .... 102.5		" 24 .... 102.0
" 11 .... 102.6		" 25 .... 102.2

Result: Guinea-pig 36 did not develop typhus during an incubation period of thirty-eight days, after inoculation with the viscera of louse 1 of Box XXXIV. After inoculation with blood from a typhus patient, it failed also to develop typhus during a further observation period of twenty-six days.

The results are inconclusive in that the failure to develop typhus following the injection of human blood is compatible with the common experiences with normal guinea-pigs and is not definite proof of immunity.

Conclusion: Rickettsia-free louse 1 of Box xxxiv probably did not contain the virus of typhus.

Record of Guinea-pig 37, inoculated with the viscera of louse 2 of Box XXXIV

Temperatures following the inoculation on May 22d:

	°F.		°F.
May 22	No record	June 10	102.0
" 23	102.6	" 11	101.2
" 24	102.2	" 12	101.4
" 25	103.0	" 13	102.0
" 26	103.4	" 14	103.4
" 27	102.0	" 15	103.0
" 28	102.8	" 16	102.6
" 29	103.0	" 17	No record
" 30	102.6	" 18	103.4
" 31	102.0	" 19	103.0
June 1	103.0	" 20	No record
" 2	102.0	" 21	103.0
" 3	103.2	" 22	No record
" 4	101.6	" 23	102.7
" 5	102.3	" 24	No record
" 6	102.2	" 25	102.8
" 7	102.6	" 26	No record
" 8	102.0	" 27	102.2
" 9	101.8		

On June 28th this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 814 (eighth day of typhus) and the subsequent temperatures were as follows:

	°F.		°F.
June 28	102.5	July 8	102.1
" 29	No record	" 9	No record
" 30	102.4	" 10	102.3
July 1	101.6	" 11	102.2
" 2	No record	" 12	103.1
" 3	105.1	" 13	102.7
" 4	103.1	" 14	106.0
" 5	103.4	" 15	No record
" 6	No record	" 16	105.2
" 7	103.1	" 17	104.5

July 17th; killed. The autopsy showed no lesions. The tissues were lost, and no histological examination could be made.

Result: Guinea-pig 37, following inoculation with the viscera of louse 2 of Box xxxiv, did not develop typhus during an observation period of thirty-eight days.

Following an injection of human typhus blood, after an incubation period of sixteen days, it developed temperatures consistent with typhus.

Conclusion: Rickettsia-free louse 2 of Box xxxiv did not contain the virus of typhus.

*Record of Guinea-pig 38, inoculated with the viscera of
louse 3 of Box XXXIV*

The temperatures following the inoculation on May 22d were as follows:

	°F.		°F.
May 22 .... No record		June 10 .... 103.0	
" 23 .... 104.0		" 11 .... 102.0	
" 24 .... 102.9		" 12 .... 102.2	
" 25 .... 103.2		" 13 .... 102.4	
" 26 .... 103.3		" 14 .... 103.0	
" 27 .... 103.8		" 15 .... 103.6	
" 28 .... 102.4		" 16 .... 103.4	
" 29 .... 102.2		" 17 .... 103.0	
" 30 .... 102.7		" 18 .... 102.6	
" 31 .... 102.4		" 19 .... 102.9	
June 1 .... 102.2		" 20 .... No record	
" 2 .... 102.3		" 21 .... 104.2	
" 3 .... 102.2		" 22 .... No record	
" 4 .... 102.6		" 23 .... 103.6	
" 5 .... 102.4		" 24 .... No record	
" 6 .... 102.0		" 25 .... 103.6	
" 7 .... 102.8		" 26 .... No record	
" 8 .... 103.0		" 27 .... 103.8	
" 9 .... 103.0			

On June 28th this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 814 (eighth day of typhus). The subsequent temperatures were as follows:

		°F.				°F.
June	28	103.0	July	8		102.1
"	29	No record	"	9		No record
"	30	102.6	"	10		102.6
July	1	103.0	"	11		102.8
"	2	No record	"	12		102.4
"	3	104.7	"	13		102.3
"	4	103.8	"	14		104.4
"	5	103.2	"	15		No record
"	6	No record	"	16		104.0
"	7	102.7	"	17		103.8

July 18th; killed. The autopsy showed an enlarged spleen covered with a thin transparent fibrin-like layer. The mesenteric lymph nodes were enlarged and caseous (pseudo-tuberculosis).

Result: In view of the pseudo-tuberculosis found at autopsy, it is evident that the irregular elevations of temperature during the thirty-eight days of observation after the louse injection were not due to typhus. On the other hand, the onset of temperature sixteen days after the injection of human typhus blood is consistent with typhus.

Conclusion: Rickettsia-free louse 3 of Box xxxiv did not contain the virus of typhus.

Record of Guinea-pig 39, inoculated with louse 4 of Box XXXIV

Temperatures following the inoculation on May 22d:

		°F.			°F.
May	22	No record	June 10		102.8
"	23	104.0	" 11		103.0
"	24	102.9	" 12		102.4
"	25	104.0	" 13		102.8
"	26	104.1	" 14		103.6
"	27	103.2	" 15		102.8
"	28	102.2	" 16		102.9
"	29	102.8	" 17		103.8
"	30	102.7	" 18		104.2
"	31	102.2	" 19		103.2
June	1	103.2	" 20		No record
"	2	103.0	" 21		103.3
"	3	101.9	" 22		No record
"	4	102.0	" 23		102.7
"	5	102.0	" 24		No record
"	6	101.8	" 25		102.4
"	7	103.2	" 26		No record
"	8	103.0	" 27		103.0
"	9	102.8			

On June 28th this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 814 (eighth day of typhus). The subsequent temperatures were as follows:

	°F.		°F.
June 28 .... 102.8		July 8 .... 104.1	
" 29 .... No record		" 9 .... No record	
" 30 .... 103.6		" 10 .... 104.1	
July 1 .... 103.6		" 11 .... 102.6	
" 2 .... No record		" 12 .... 101.7	
" 3 .... 103.9		" 13 .... 104.2	
" 4 .... No record		" 14 .... 104.4	
" 5 .... 104.1		" 15 .... No record	
" 6 .... No record		" 16 .... 103.3	
" 7 .... 104.9		" 17 .... 102.4	

July 17th; killed. The autopsy showed massive lesions of pseudo-tuberculosis in the lymph nodes of the mesentery. The spleen was moderately enlarged. As the tissues were lost, there are no histological data.

Result: At no time in the thirty-eight days following the louse injection did this guinea-pig have a course of temperature consistent with typhus. A period of temperature followed the test for immunity with typhus blood consistent with typhus.

Conclusion: Rickettsia-free louse 4 of Box xxxiv probably did not contain the virus of typhus.

Experiments with Box XXXVI lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 30° C., usually 29° C. The box contained 30 W lice (30 nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
May 8............	....	6.00	Placed on Case 110	8th
" 9............	12.30	9.15	" " "	9th
" 10............	12.53	....	" " "	10th
" 11............	12.30	6.45	" " "	11th
" 12............	10.40	....	" " "	12th
" 13............	11.20	....	" " "	13th
" 14............	12.00	....	" " "	14th
" 15............	....	1.00	" " "	15th
" 16............	11.30	6.30	Transferred to Case 121	14th
" 17............	11.00	7.00	" " "	15th
" 18............	10.30	7.30	" " "	16th
" 19............	12.00	....	" " "	17th
" 20............	10.30	7.30	" " "	18th
" 21............	11.30	7.30	" " 132	12th
" 22............	11.00	2.00	" " 125	14th
" 24............	....		Box removed, kept at 20° to 30° C. usually 29° C. until box opened; it contained a little feces, 3 eggs, no living nymphs or larvae, 11 living adult lice.	

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears		
1	Rickettsia absent	Guinea-pig 40; Temp. — Proved susceptible to blood from a typhus patient
Sections		
1	Rickettsia absent	

*Record of Guinea-pig 40, inoculated with the viscera of
louse 1 of Box XXXVI*

The temperatures following the inoculation on May 24th
were as follows:

	°F.			°F.
May 24	102.4		June 11	101.0
" 25	102.2		" 12	101.4
" 26	101.8		" 13	101.0
" 27	102.4		" 14	102.0
" 28	101.8		" 15	102.2
" 29	102.2		" 16	101.8
" 30	102.2		" 17	No record
" 31	102.4		" 18	102.4
June 1	101.8		" 19	102.6
" 2	102.2		" 20	No record
" 3	101.8		" 21	102.0
" 4	101.8		" 22	No record
" 5	101.0		" 23	102.4
" 6	102.0		" 24	No record
" 7	102.0		" 25	No record
" 8	102.0		" 26	No record
" 9	101.8		" 27	102.8
" 10	102.0			

On June 28th this guinea-pig was inoculated intraperito-
neally with 5 c.c. of blood from patient 814 (eighth day of
typhus).

The subsequent temperatures were as follows:

	°F.			°F.
June 28	102.9		July 12	102.4
" 29	No record		" 13	102.9
" 30	102.7		" 14	103.7
July 1	102.6		" 15	No record
" 2	No record		" 16	103.6
" 3	104.2		" 17	103.6
" 4	102.8		" 18	103.8
" 5	102.4		" 19	103.2
" 6	No record		" 20	102.7
" 7	102.1		" 21	No record
" 8	102.1		" 22	102.6
" 9	No record		" 23	102.4
" 10	102.3		" 24	102.1
" 11	102.6		" 25	101.7

There is no record of an autopsy.

' *Result:* Guinea-pig 40 did not develop typhus during an observation period of thirty-six days following inoculation with the viscera of louse 1 of Box XXXVI. Fifteen days following the injection of human blood from patient 814 it developed a moderately elevated temperature lasting for six days, followed by normal temperatures.

Conclusion: Louse 1 of Box XXXVI did not contain the virus of typhus.

Experiments with Box XXXVII lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 30° C., usually 29° C. The box contained 30 W lice (30 nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
May 10............	12.00	....	Placed on Case 100	18th
" 11............	12.30	6.15	" " "	19th
" 12............	10.18	....	" " "	20th
" 13............	11.00	....	" " "	21st
" 14............	12.00	....	" " "	22d
" 15............	....	1.00	" " "	23d
" 16............	11.45	7.30	" " "	....
" 17............	11.30	....	Transferred to Case 127	11th
	....	6.30	" " 125	9th
" 18............	10.30	7.30	" " "	10th
" 19............	12.00	....	" " 131	9th
" 20............	10.30	7.30	" " "	10th
" 21............	11.30	7.30	" " "	11th
" 22............	11.00	2.00	" " "	12th
" 24............	....		Box removed, kept at 20° to 30° C. usually 29° C. until box opened; it contained much feces, some eggs, no nymphs or larvae, 23 living adult lice.	

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears		
1	Rickettsia absent	Guinea-pig 41; Temp. — Proved susceptible to blood from a typhus patient
2	Rickettsia absent	Guinea-pig 42; Temp. — Proved susceptible to blood from a typhus patient
3	Rickettsia absent	Guinea-pig 43; Temp. — Proved susceptible to blood from a typhus patient
4	Rickettsia absent	Guinea-pig 44; Temp. — Proved susceptible to blood from a typhus patient
14	Rickettsia absent	
15	" "	
16	" "	
17	" "	
18	" "	
19	" "	
20	" "	
21	" "	
Nymph	" "	
Excreta	" "	
Sections		
W 1	Rickettsia absent	
W 2	" "	
4	" "	
6	" "	

*Record of Guinea-pig 41, inoculated with the viscera of
louse 1 of Box XXXVII*

The temperatures following the inoculation on May 24th
were as follows:

	°F.			°F.
May 24	102.6		June 4	102.0
" 25	102.0		" 5	102.2
" 26	103.7		" 6	102.2
" 27	No record		" 7	101.4
" 28	102.8		" 8	102.0
" 29	102.6		" 9	101.4
" 30	102.4		" 10	102.0
" 31	102.0		" 11	101.8
June 1	102.3		" 12	102.0
" 2	102.6		" 13	101.6
" 3	102.2		" 14	102.0

		°F.				°F.
June	15	102.0	June	22		No record
"	16	No record	"	23		102.0
"	17	No record	"	24		No record
"	18	102.7	"	25		102.5
"	19	102.4	"	26		No record
"	20	No record	"	27		102.7
"	21	101.8				

On June 28th this guinea-pig was inoculated with 5 c.c. of blood from patient 814 (eighth day of typhus). The subsequent temperatures were as follows:

		°F.			°F.
June	28	102.0	July	12	102.0
"	29	No record	"	13	102.6
"	30	102.6	"	14	103.4
July	1	101.7	"	15	No record
"	2	No record	"	16	103.4
"	3	105.3	"	17	103.6
"	4	103.0	"	18	103.4
"	5	102.5	"	19	103.4
"	6	No record	"	20	103.6
"	7	102.1	"	21	No record
"	8	101.5	"	22	103.6
"	9	No record	"	23	103.2
"	10	101.8	"	24	102.9
"	11	102.0	"	25	102.6

There is no record of an autopsy.

Result: Guinea-pig 41, following inoculation with the viscera of louse 1 of Box xxxvii, during an observation period of thirty-four days, did not develop typhus. Sixteen days after the inoculation with blood from a typhus patient it developed moderately high temperatures lasting ten days. The coincidence of the temperature elevation with that of other guinea-pigs injected with the blood of patient 814 on June 28th strengthens the evidence that typhus followed the blood injection.

Conclusion: Louse 1 of Box xxxvii did not contain the virus of typhus.

Record of Guinea-pig 42, inoculated with the viscera of louse 2 of Box XXXVII

Temperatures following the inoculation on May 24th:

	°F.			°F.
May 24	102.2		June 11	101.0
" 25	102.7		" 12	101.2
" 26	102.6		" 13	101.4
" 27	102.8		" 14	101.0
" 28	102.4		" 15	101.2
" 29	102.0		" 16	No record
" 30	102.4		" 17	No record
" 31	102.8		" 18	102.1
June 1	102.6		" 19	102.4
" 2	102.2		" 20	No record
" 3	102.4		" 21	101.4
" 4	101.8		" 22	No record
" 5	102.4		" 23	102.1
" 6	101.8		" 24	No record
" 7	101.0		" 25	102.2
" 8	101.4		" 26	No record
" 9	101.6		" 27	102.0
" 10	101.8			

On June 28th this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 814 (eighth day of typhus). The subsequent temperatures were as follows:

	°F.			°F.
June 28	102.1		July 8	102.5
" 29	No record		" 9	No record
" 30	102.2		" 10	102.4
July 1	102.4		" 11	102.6
" 2	No record		" 12	102.4
" 3	104.8		" 13	104.8
" 4	102.4		" 14	104.6
" 5	102.0		" 15	No record
" 6	No record		" 16	104.4
" 7	102.2		" 17	104.1

July 17th; killed. The autopsy was negative except for an enlarged spleen covered with a thin transparent fibrin-like layer. The tissues were lost, and no histological data were obtained.

Result: Guinea-pig 42, inoculated with the viscera of louse 2 of Box xxxvii, during an observation period of thirty-four days, did not develop typhus. Fifteen days after inoculation with human typhus blood it developed, coincidently with others receiving the same blood, the reaction of typhus.

Conclusion: Louse 2 of Box xxxvii did not contain the virus of typhus.

*Record of Guinea-pig 43, inoculated with the viscera of
louse 3 of Box XXXVII*

Temperatures following the inoculation on May 24th:

	°F.			°F.
May 24	102.5	June 11		101.8
" 25	102.4	" 12		101.2
" 26	102.4	" 13		101.8
" 27	102.6	" 14		102.0
" 28	102.8	" 15		101.8
" 29	103.0	" 16		No record
" 30	102.9	" 17		No record
" 31	102.0	" 18		102.5
June 1	101.8	" 19		102.0
" 2	102.0	" 20		No record
" 3	101.9	" 21		102.6
" 4	101.8	" 22		No record
" 5	102.6	" 23		102.3
" 6	102.2	" 24		No record
" 7	102.4	" 25		102.4
" 8	101.8	" 26		No record
" 9	102.0	" 27		102.0
" 10	101.8			

On June 28th this guinea-pig was inoculated intraperitone-
ally with 5 c.c. of blood from patient 814 (eighth day of ty-
phus). The subsequent temperatures were as follows:

	°F.			°F.
June 28	102.5	July 8		102.8
" 29	No record	" 9		No record
" 30	102.4	" 10		103.4
July 1	102.8	" 11		102.8
" 2	No record	" 12		103.1
" 3	103.8	" 13		104.9
" 4	103.2	" 14		105.0
" 5	103.1	" 15		No record
" 6	No record	" 16		104.8
" 7	102.9	" 17		104.2

July 17th; killed. The autopsy was negative except for an
enlarged spleen covered with a thin transparent layer of
fibrin-like material.

Result: Guinea-pig 43, inoculated with the viscera of louse 3
of Box xxxvii, during an observation period of thirty-four
days did not develop typhus. Fifteen days after inoculation
with human typhus blood it developed, coincidently with
others receiving the same blood, the reaction of typhus.

Conclusion: Louse 3 of Box xxxvii did not contain the
virus of typhus.

Record of Guinea-pig 44, inoculated with the viscera of
louse 4 of Box XXXVII

The temperatures following the inoculation on May 24th
were as follows:

	°F.		°F.
May 24	102.0	June 11	102.0
" 25	102.2	" 12	102.4
" 26	102.9	" 13	102.2
" 27	103.0	" 14	102.0
" 28	102.4	" 15	102.4
" 29	102.0	" 16	No record
" 30	102.7	" 17	No record
" 31	103.0	" 18	102.4
June 1	103.0	" 19	102.3
" 2	102.6	" 20	No record
" 3	102.4	" 21	102.7
" 4	101.8	" 22	No record
" 5	102.6	" 23	102.5
" 6	102.4	" 24	No record
" 7	102.6	" 25	102.6
" 8	102.0	" 26	No record
" 9	101.8	" 27	102.6
" 10	102.0		

On June 28th this guinea-pig was inoculated intraperitone-
ally with 5 c.c. of blood from patient 814 (eighth day of ty-
phus). The subsequent temperatures were as follows:

	°F.		°F.
June 28	102.0	July 12	102.6
" 29	No record	" 13	103.1
" 30	102.4	" 14	103.2
July 1	102.3	" 15	No record
" 2	No record	" 16	102.2
" 3	105.1	" 17	102.0
" 4	102.9	" 18	102.1
" 5	103.2	" 19	102.2
" 6	No record	" 20	101.8
" 7	102.1	" 21	No record
" 8	102.2	" 22	101.8
" 9	No record	" 23	102.0
" 10	102.7	" 24	102.0
" 11	102.8	" 25	102.0

There is no record of an autopsy.

Result: Guinea-pig 44, inoculated with the viscera of louse 4
of Box xxxvii, during an observation period of thirty-four
days did not develop typhus fever. During a further observa-

tion period of twenty-eight days, following an inoculation with human blood on June 28th, it failed to react, although on July 13th and 14th there was a slight rise of temperature coincident with the onset of typhus in other guinea-pigs receiving the same blood on the same date.

Conclusion: Louse 4 of Box xxxvii probably did not contain the virus of typhus.

Experiments with Box XXXVIII lice

Between feedings the box was kept in an incubator at a temperature varying from 20° to 30° C., usually 29° C. The box contained 30 W lice (30 nymphs).

1920	Fed for from one-half to one hour at		Fed on Case No.	Day of Disease
	A.M.	P.M.		
May 11..............	12.15	6.30	Placed on Case 116	4th
" 12..............	10.30	....	" " "	5th
" 13..............	11.15	....	" " "	6th
" 14..............	12.15	....	" " "	7th
" 15..............	....	1.00	" " "	8th
" 16..............	11.45	7.30	" " "	9th
" 17..............	11.30	6.30	" " "	10th
" 18..............	10.30	7.30	" " "	11th
" 19..............	12.00	....	" " "	12th
" 20..............	10.30	7.30	" " "	13th
" 21..............	11.30	7.30	" " "	14th
" 22..............	12.00	2.00	Transferred to Case 134	8th
" 24..............	....		Box removed, kept at 20° to 30° C. usually 29° C. until box opened; it contained feces, eggs, one living second stage larva, and 12 living adult lice.	

From these lice the following preparations were made:

Louse No.	Result of Microscopical Examination	Result of Animal Inoculation
Smears 1	Rickettsia absent	Guinea-pig 45; Temp. −
2	Rickettsia absent	Guinea-pig 46; Temp. + Lesions present
3	Rickettsia present	Guinea-pig 47; Temp. +
4	" "	Guinea-pig 48; Temp. +
Excreta	" absent	
Dead red male	" present	

*Record of Guinea-pig 45, inoculated with rickettsia-free
louse 1 of Box XXXVIII*

Temperatures following the inoculation on May 24th:

	°F.			°F.
May 24	102.4	June 11		102.0
" 25	102.0	" 12		102.4
" 26	103.0	" 13		102.0
" 27	102.5	" 14		102.8
" 28	102.6	" 15		103.6
" 29	102.6	" 16		No record
" 30	102.4	" 17		No record
" 31	102.0	" 18		102.0
June 1	102.2	" 19		102.4
" 2	102.4	" 20		No record
" 3	102.0	" 21		102.0
" 4	102.6	" 22		No record
" 5	102.0	" 23		102.7
" 6	102.0	" 24		No record
" 7	102.0	" 25		102.4
" 8	101.8	" 26		No record
" 9	101.4	" 27		102.8
" 10	101.4			

On June 28th this guinea-pig was inoculated intraperitone-
ally with 5 c.c. of blood from patient 814 (eighth day of ty-
phus) and the subsequent temperatures were as follows:

	°F.			°F.
June 28	102.5	July 8		101.8
" 29	No record	" 9		No record
" 30	102.7	" 10		101.9
July 1	102.7	" 11		102.2
" 2	No record	" 12		102.4
" 3	104.4	" 13		104.9
" 4	103.4	" 14		105.3
" 5	103.0	" 15		No record
" 6	No record	" 16		105.0
" 7	102.2	" 17		104.6

July 17th; killed. The autopsy was negative except for a
much enlarged spleen covered with a thin transparent fibrin-
like layer. The tissues were lost, and no histological data were
obtained.

Result: Guinea-pig 45, inoculated with the viscera of
rickettsia-free louse 1 of Box xxxviii did not develop typhus
during an observation period of thirty-four days. After a further
observation period of fifteen days, following an inoculation with

human typhus blood on June 28th, it developed typhus, coincident with other guinea-pigs inoculated with the same blood.

Conclusion: Rickettsia-free louse 1 of Box xxxviii did not contain the virus of typhus.

Record of Guinea-pig 46, inoculated with the viscera of rickettsia-free louse 2 of Box XXXVIII

Temperatures following the inoculation on May 24th:

	°F.			°F.
May 24	102.6	June	2	102.2
" 25	102.2	"	3	102.2
" 26	103.3	"	4	103.0
" 27	102.2	"	5	102.8
" 28	102.2	"	6	105.2
" 29	102.0	"	7	104.0
" 30	102.6	"	8	104.3
" 31	102.2	"	9	104.2
June 1	102.0			

June 9th; killed. The autopsy showed a spleen of twice the normal size, without exudate. All other tissues were negative. Histological examination showed the characteristic lesions of typhus in the brain.

Two guinea-pigs, Nos. 74 and 75, were inoculated, each intraperitoneally, with 3 c.c. of blood from the heart of this guinea-pig 46.

The temperatures of these two guinea-pigs follow:

	No. 74 °F.	No. 75 °F.			No. 74 °F.	No. 75 °F.
June 9	No record	No record	June 25		104.8	102.8
" 10	104.0	103.8	" 26		No record	No record
" 11	104.0	103.0	" 27		104.0	103.0
" 12	102.8	104.2	" 28		No record	No record
" 13	103.0	104.4	" 29		102.8	103.0
" 14	103.6	103.0	" 30		103.8	103.0
" 15	103.0	102.0	July 1		102.9	102.0
" 16	No record	No record	" 2		No record	No record
" 17	No record	No record	" 3		104.3	103.6
" 18	103.6	102.8	" 4		104.6	103.0
" 19	103.8	102.8	" 5		104.5	102.8
" 20	No record	No record	" 6		No record	No record
" 21	104.6	102.8	" 7		104.2	102.4
" 22	No record	No record	" 8		102.0	101.8
" 23	105.4	102.2	" 9		No record	No record
" 24	No record	102.8	" 10		102.2	102.1

Result: Guinea-pig 46, inoculated with louse 2 of Box xxxviii, in a preparation of which no rickettsia were found, developed a course of temperature consistent with typhus fever. The infection was proved to be typhus by histological examination. One of two guinea-pigs inoculated with blood from guinea-pig 46 developed a reaction consistent with typhus.

Conclusion: Louse 2 of Box xxxviii, in a preparation of which no rickettsia were found, contained the virus of typhus.

Record of Guinea-pig 47, inoculated with rickettsia-infected louse 3 of Box XXXVIII

The temperatures following the inoculation on May 24th were as follows:

		°F.				°F.
May	24	102.5	June	11		103.6
"	25	102.6	"	12		103.8
"	26	103.2	"	13		104.0
"	27	103.0	"	14		102.8
"	28	103.0	"	15		102.0
"	29	103.2	"	16		No record
"	30	103.0	"	17		No record
"	31	102.6	"	18		103.0
June	1	102.0	"	19		102.4
"	2	104.0	"	20		No record
"	3	103.8	"	21		102.7
"	4	103.0	"	22		No record
"	5	105.0	"	23		102.4
"	6	104.8	"	24		No record
"	7	104.0	"	25		102.2
"	8	104.0	"	26		No record
"	9	104.0	"	27		102.2
"	10	104.0				

On June 28th this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 814 (eighth day of typhus) and the subsequent temperatures were as follows:

		°F.				°F.
June	28	102.4	July	12	101.6
"	29	No record	"	13	102.4
"	30	102.5	"	14	103.1
July	1	102.6	"	15	No record
"	2	No record	"	16	102.4
"	3	104.3	"	17	102.1
"	4	103.2	"	18	102.2
"	5	103.4	"	19	103.0
"	6	No record	"	20	102.4
"	7	102.3	"	21	No record
"	8	101.4	"	22	102.1
"	9	No record	"	23	101.7
"	10	102.6	"	24	102.6
"	11	102.3	"	25	102.0

Result: Guinea-pig 47, inoculated with the viscera of rickettsia-infected louse 3 of Box xxxviii, developed typhus after an incubation period of ten to thirteen days.

It failed to react after inoculation on June 28th with human typhus blood known to be infectious, during an observation period of twenty-eight days.

Conclusion: Rickettsia-infected louse 3 of Box xxxviii contained the virus of typhus.

Record of Guinea-pig 48, inoculated with rickettsia-infected louse 4 of Box XXXVIII

Temperatures following the inoculation on May 24th:

		°F.				°F.
May	24	102.2	June	11	102.2
"	25	102.5	"	12	102.0
"	26	104.8	"	13	102.8
"	27	105.2	"	14	103.0
"	28	104.6	"	15	102.6
"	29	103.6	"	16	No record
"	30	103.8	"	17	No record
"	31	103.6	"	18	102.6
June	1	103.6	"	19	102.7
"	2	103.2	"	20	No record
"	3	103.0	"	21	103.0
"	4	103.6	"	22	No record
"	5	104.3	"	23	102.7
"	6	104.4	"	24	No record
"	7	104.2	"	25	102.4
"	8	103.8	"	26	No record
"	9	103.4	"	27	102.6
"	10	103.6			

On June 28th this guinea-pig was inoculated intraperitoneally with 5 c.c. of blood from patient 814 (eighth day of typhus). The subsequent temperatures were as follows:

	°F.			°F.
June 28	102.5	July 12		103.1
" 29	No record	" 13		104.1
" 30	102.6	" 14		103.6
July 1	103.6	" 15		No record
" 2	No record	" 16		102.2
" 3	104.4	" 17		102.1
" 4	105.2	" 18		102.1
" 5	104.8	" 19		102.6
" 6	No record	" 20		102.2
" 7	103.0	" 21		No record
" 8	102.4	" 22		102.1
" 9	No record	" 23		102.1
" 10	102.7	" 24		102.6
" 11	102.6	" 25		102.4

There is no record of an autopsy.

Result: Following the inoculation with the viscera of rickettsia-infected louse 4 of Box xxxviii, this guinea-pig 48 had two periods of elevated temperature, the second one corresponding in incubation period and duration with the temperatures of typhus. After the injection of human typhus blood there followed a rise of temperature on the fourth day, the duration of which was influenced by the conditions of July 3d. No other period of temperature occurred except that of July 13th and 14th, which corresponded to the usual incubation of typhus in guinea-pigs inoculated with the blood of patient 814, but the course was not that of typhus; nevertheless the immunity of guinea-pig 48 may be open to question.

Conclusion: Rickettsia-infected louse 4 of Box xxxviii contained the virus of typhus.

3. SUMMARY OF THE EXPERIMENTS TO PROVE THE SPECIFICITY OF *RICKETTSIA PROWAZEKI* FOR TYPHUS

These experiments are reviewed in Table IX.

In four of the boxes used for these experiments, Nos. XVII, XVIII, XIX, and XXXVIII, lice containing *Rickettsia prowazeki* were found. In six of the boxes, Nos. XXI, XXII, XXVIII, XXXIV, XXXVI, and XXXVII, no lice were found to contain rickettsia.

1. From the four positive boxes, each of the nine lice in which rickettsia were found produced typhus in guinea-pigs. The existence of typhus was proved in every instance but one (louse W 241, guinea-pig 22) by adequate controls.

2. From the four positive boxes, there were four lice in which no rickettsia were found; but they produced typhus in guinea-pigs. In each instance the existence of typhus was proved by adequate controls. The failure to find rickettsia in smears of such small amounts as were reserved for examination in these inoculation experiments does not prove their absence from the lice; for, in the examination of serial sections of entire lice from positive boxes, we occasionally have found rickettsia in but a single cell.

3. From the four positive boxes, there were seven lice in which no rickettsia were found; they failed to produce typhus in guinea-pigs. The failures were proved by adequate controls in every instance but one (louse W 240, Box XVII); this animal lived for forty days without evidence of typhus.

4. One injection from a negative louse from a positive box (louse W 223, Box XVIII, guinea-pig 24) yielded inconclusive results in that the guinea-pig, after a period of temperature, died on the thirteenth day of pneumonia.

5. From the negative boxes, no louse produced typhus in a guinea-pig. Of the total of twenty guinea-pigs injected with lice viscera from these boxes, the results were adequately controlled in twelve, and not controlled in one instance (louse W 240, Box XVII, guinea-pig 21) in which case, however, the guinea-pig lived for forty days without evidence of typhus. Three experiments were failures owing to the premature death

4. TABLE IX. TABLE OF EXPERIMENTS IN PROOF OF THE SPECIFICITY OF *RICKETTSIA PROWAZEKI* FOR TYPHUS

Guinea-pig No.	Louse Box No.	Louse No.	Rick-ettsia	Result of Guinea-pig Inoculation	Control of Guinea-pig Reaction
7	xix	male W 212	—	—	Susceptibility to human typhus blood proved
8	xix	fem. W 214	—	+	Proved to be immune to human typhus blood
9	xviii	fem. W 227	+	+	Questionable reaction to human typhus blood. Probably was immune
10	xviii	fem. W 226	—	—	Susceptibility to human typhus blood proved
11	xvii	fem. W 236	—	—	Susceptibility to human typhus blood proved
12	xix	fem. W 216	—	—	Susceptibility to human typhus blood proved
13	xix	male W 211	+	+	Proved to be resistant to human typhus blood
14	xix	fem. W 213	—	+	Proved by histology
15	xviii	fem. W 228	+	+	Proved by histology
16	xviii	fem. W 225	—	—	Susceptibility to human typhus blood proved
17	xvii	fem. W 237	—	—	Susceptibility to human typhus blood proved
18	xix	nym. W 215	—	+	Proved by histology and subsequent guinea-pig inoculation
19	xvii	fem. W 239	+	+	Proved by histology and subsequent guinea-pig inoculation
20	xvii	fem. W 238	+	+	Proved by histology and subsequent guinea-pig inoculation
21	xvii	fem. W 240	—	—	No control; died 40 days
22	xvii	fem. W 241	+	+	No control; died 40 days
23	xviii	fem. W 224	+	+	Proved by histology and subsequent guinea-pig inoculation
24	xviii	male W 223	—	+	No control; died of pneumonia in 13 days
25	xxi	fem. W 261	—	Died 3d day	Pneumonia
26	xxi	fem. W 262	—	—?	Autopsy and histology negative *
27	xxi	fem. W 263	—	—	Autopsy negative; acute enteritis
28	xxi	male W 264	—	—	Susceptibility to human typhus blood proved
29	xxii	male W 265	—	—	Did not react to human typhus blood. Outcome of experiment doubtful

* Blood proved not infectious for guinea-pig 72. Immunity test of guinea-pig 72 unsatisfactory.

Guinea-pig No.	Louse Box No.	Louse No.	Rick-ettsia	Result of Guinea-pig Inoculation	Control of Guinea-pig Reaction
30	xxii	male W 266	—	—	Susceptibility to human typhus blood proved
31	xxii	male W 267	—	—	Died in 20 days — pneumonia
32	xxviii	male W 1	—	—	Susceptibility to human typhus blood proved
33	xxviii	fem. W 2	—	Died 10 days	Pneumonia
34	xxviii	male W 3	—	—	Susceptibility to human typhus blood proved
35	xxviii	fem. W 4	—	Died 7 days	Pseudo-tuberculosis
36	xxxiv	male W 1	—	—	Did not react to human typhus blood
37	xxxiv	fem. W 2	—	—	Susceptibility to human typhus blood proved
38	xxxiv	male W 3	—	—	Susceptibility to human typhus blood proved
39	xxxiv	fem. W 4	—	—	Susceptibility to human typhus blood proved
40	xxxvi	fem. W 1	—	—	Susceptibility to human typhus blood proved
41	xxxvii	fem. W 1	—	—	Susceptibility to human typhus blood proved
42	xxxvii	male W 2	—	—	Susceptibility to human typhus blood proved
43	xxxvii	fem. W 3	—	—	Susceptibility to human typhus blood proved
44	xxxvii	fem. W 4	—	—	Did not react to human typhus blood
45	xxxviii	male W 1	—	—	Susceptibility to human typhus blood proved
46	xxxviii	fem. W 2	—	+	Proved by histology and subsequent guinea-pig inoculation
47	xxxviii	male W 3	+	+	Proved to be resistant to human typhus blood
48	xxxviii	male W 4	+	+	Proved to be resistant to human typhus blood

of the guinea-pigs. The results of four inoculation experiments, although the guinea-pigs did not develop typhus, may be regarded as open to question. Guinea-pigs 29, 36, 39, and 44 inoculated respectively with louse W 265, Box xxii, louse W 1, Box xxxiv, louse 4, Box xxxiv, and louse 4, Box xxxvii, while not reacting to the louse injections, did not react to the blood of a typhus patient.

Conclusions

The presence of *Rickettsia prowazeki* in lice in our experience is proof of the presence of the virus of typhus.

A variable percentage only of lice nurtured upon typhus patients acquire the virus of typhus; and this holds true in boxes where all lice have equal opportunities to become infected.

After allowing for the technical difficulties in making adequate search for rickettsia and injections from the same louse and for the uncertainty of the reaction of guinea-pigs to typhus blood in the control inoculations in the test for immunity, we believe that the data from the above experiments are sufficient proof that the virus of typhus and *Rickettsia prowazeki* are inseparable.

VII

CULTIVATION EXPERIMENTS

1. TECHNIC

THE technic described by Plotz, Olitsky, and Baehr (1915) in their monograph on the etiology of typhus was minutely followed. We had ascitic fluid from only one source for these experiments; but this ascitic fluid was sterile, free from bile and had a specific gravity of 1020. It did not inhibit the growth of other bacteria.

The steps of the procedure were as follows:

1. The agar in test tubes of the diameter specified by Plotz was melted and cooled to 40° C.

2. Thirty cubic centimeters of blood was drawn under aseptic conditions and two and a half cubic centimeters was added to each of three tubes, each containing twenty cubic centimeters of 2.5 per cent glucose agar.

3. Four cubic centimeters of ascitic fluid was added to each of the tubes containing glucose agar and blood.

4. As controls to the manipulative part of the procedure four cubic centimeters of ascitic fluid was added to each of three tubes of the same agar to which no blood had been added.

5. The contents of the tubes, cultures, and controls were mixed by pouring back and forth into sterile test tubes.

6. The cultures and control tubes of agar were hardened in a vessel of ice-water.

7. Four cubic centimeters of plain agar were added to each tube of hardened medium.

2. CASES

In addition to the anaerobic cultures as prepared, we made from each case aerobic cultures by adding the blood to glucose broth in flasks and by plating in plain agar and in glucose agar.

The cultures were made from fourteen cases as follows:

Case 130	5th day of typhus	Case 162 ...... 7th day of typhus
" 135 ...... 7th " " "		" 163 ...... 7th " " "
" 138 ...... 11th " " "		" 165 ...... 9th " " "
" 142 ...... 8th " " "		" 171 ...... 9th " " "
" 144 ...... 8th " " "		" 182 ...... 11th " " "
" 149 ...... 8th " " "		" 180 ...... day of disease un-
" 152 ...... 8th " " "		known, second week?
" 158 ...... 6th " " "		

The Plotz cultures were examined daily by transmitted light, and each tube before being discarded was broken and searched for colonies by sectioning the medium.

3. RESULTS OF THE ANAEROBIC CULTURES

Five cases only showed no growth. The shortest time of observation was nine days, the longest thirty days. No growth occurred in control tubes made coincidentally with these five cultures.

Six culture tubes from six cases yielded growth of bacteria; from three cases a large gram-positive bacillus; from one case a gram-positive diplococcus; from one case a large gram-negative bacillus; and, in one case, a single colony which occurred in one tube was not examined.

The control cultures of ascitic fluid in six instances yielded growth. In four, a large gram-positive bacillus, presumably the same as that in the blood cultures, in one instance a gram-positive micrococcus and in one case a gram-negative bacillus.

The large gram-positive bacillus in all of the blood cultures and control tubes developed late in the media.[1]

4. CONTROL AEROBIC CULTURES

Cultures from Cases 138 and 165 were positive. From Case 138 a gram-positive diplococcus developed in plates and was apparently the same organism that developed in the anaerobic

[1] Kuczynski, in July, 1920, published an account of the cultivation of *Rickettsia prowazeki* from the brains of typhus infected guinea-pigs. He employed a medium of citrated blood plasma to which was added blood treated with dilute sulphuric acid and neutralized in order to supply the products of protein decomposition. The cultures were grown in collodian sacs in the peritoneal cavities of guinea-pigs. We have not yet attempted to repeat his procedures.

cultures from this case. From Case 165 a short chained streptococcus developed in the flask of glucose broth.

Results: Under presumably favorable conditions, we failed in fourteen attempts to cultivate from typhus patients a bacillus resembling that described by Plotz.

ADDITIONAL AEROBIC BLOOD CULTURES

Blood cultures were taken on eight cases, to determine the frequency of cases with which terminal invasions of the blood stream occurred. The cultures were taken on the following cases: Nos. 96, 87, 83, 106, 109, 113, 157 (blood cultures were made in this case on two successive days). These were all cases in critical condition and, in the majority of instances, moribund.

The media used were glucose broth, glucose agar, and plain agar. In a few of the cases, bile media was used in addition.

Results: All the cultures were negative except that from No. 157. The first culture in this case showed three distinct types of organisms present: a large gram-positive bacillus; a very small gram-negative bacillus; and a gram-positive coccus that proved to be staphylococcus albus. The culture from this case was repeated and the same types of organisms were recovered again.

VIII

RICKETTSIA

1. Historical Review

"Rickettsia" is the group name given by da Rocha-Lima (1916[2]) to minute micro-organisms with certain peculiarities found in lice. The name honors the memory of Howard Taylor Ricketts who first described micro-organisms possibly of this type in connection with studies upon typhus (Ricketts and Wilder, 1910).

Ricketts' report (1910) was meagre and his death from typhus while making his investigations in Mexico City prevented the publication of his complete observations and conclusions. Ricketts wrote as follows:

"(1) In the stained (Giemsa) preparation of the blood of patients, taken on from the seventh to the twelfth day of the disease, we invariably have found a short bacillus which has roughly the morphology of those which belong to the 'hemorrhagic septicaemia group.' Usually it appears to stain solidly, but on minute examination an unstained or faintly stained bar is seen to extend across the middle. Occasionally two organisms are seen end to end. Exact measurements have not been made, but when compared with the size of the erythrocyte, their length is estimated hardly more than two micromillimeters, and their diameter at about one-third this figure. Certain other bodies, the identity of which is not so clear, may represent degeneration or involution forms of the above. They consist of two stained granules, connected by an 'intermediate substance' which is stained faintly blue or not at all. Frequently one of these granules or 'rods' is larger than the other and stained a deep purple, whereas the small one takes a faint blue color."

"(2) In moist preparations of the blood of patients, bacillary bodies, with a structure like that mentioned above, have

been encountered in all cases. The differentiation of the forms into two bodies, separated by a line or narrow zone of a substance of different refractive power, may be observed. They possess no active motility, but vibrate more or less rapidly."

"(3) The dejecta and various organs of a large series of lice have been stained in a similar way and examined for the presence of micro-organisms. Certain groups had been deliberately infected by permitting the lice to feed upon patients, while others were supposedly normal, having been collected from healthy individuals. Streptococci, staphylococci, and oval bacilli occurring in clusters, and certain solid bacilli, are encountered irregularly and indifferently in the feces and intestinal contents of both 'normal' and 'infected' lice. Polar staining organisms have been found occasionally in the feces and intestinal contents of 'normal' lice, whereas they are present almost constantly, and often in large numbers, in similar material from 'infected' individuals."

Ricketts understood fully the fallibility of observations based upon smear preparations stained by Giemsa's stain and made every possible endeavor to eliminate error. His observations upon the occurrence of this bi-polar micro-organism in the blood has never been satisfactorily confirmed, although he estimated the number of "bacilli" as from 300 to 2000 in 0.01 c.c. of blood. We have never been able to confirm his results either with blood from Mexico typhus or from our Polish cases. It seems hardly possible that Ricketts saw preparations like those studied later by da Rocha-Lima, and others from lice, at least he does not describe the enormous numbers of micro-organisms to be found in typhus fed lice. He gives us only the following in the way of estimate: "In the intestinal contents of the 'infected' louse it is occasionally necessary to search three or four minutes before bi-polar organisms are found, but in most instances organisms of this type are much more numerous in the intestine of the 'infected' louse, and fifteen or twenty may be found in a single field."

Gavino and Gerard (1910[1]) in the same year confirmed the finding of bacilliform bodies and bi-polar bodies free in the

blood plasma of typhus cases, but only with great rarity. The former they describe as "bacilliform bodies 2μ long and $\frac{1}{2}\mu$ wide, showing at the extremities two rounded masses colored in violet-purples, the central part remaining white." The latter as "bodies $\frac{1}{2}\mu$ wide composed of two spherical corpuscles; one of the corpuscles is tinged in purple-red in the Giemsa, the other in blue."

Hegler and von Prowazek, in 1913, described appearances in neutrophilic leucocytes from fifty-one typhus cases which they believed to be micro-organisms. The bodies described by them were in the form of paired granules, resisting decolorization with alcohol after Giemsa staining and lying in vacuoles. No satisfactory confirmation of Hegler's and von Prowazek's conclusions have been offered by other workers, and from our own experience we confess inability to distinguish characteristic features of the granules to be noted in leucocytes in typhus when compared with leucocytes from other sources.

Hegler and von Prowazek (1913) mention also one instance of the finding of small coccus-like double organisms in a smear from a louse fed upon typhus cases.

A later report by von Prowazek (1915–16) again contains observations on the paired granules in leucocytes.

Sergent, Foley, and Vialette (1914) described cocco-bacilli from lice collected from typhus cases, and not in lice from persons without typhus. These organisms were described as exhibiting bi-polar staining, and as 1μ to 3μ in length and 0.5μ to 0.8μ in width. Very small forms were described as were granules. All of the forms were seen singly, in pairs, and in short chains. The proportion of lice containing these organisms and the number of organisms in the lice increased with the duration of the cases from which they were collected. They did not find these micro-organisms in thousands of lice taken from individuals without typhus. They did not attach etiological significance to these organisms and regarded them as compatible with the micro-organisms described by Ricketts and Wilder (1910[2]) and the cocci-bacilli cultivated by Galesco and Slatinearo (1906) and by M. Rabinowitsch (1909).

Nicolle, Blanc, and Conseil (1914) found cocco-bacilli like those described by Sergent, Foley, and Vialette (1914) in lice collected from a typhus free region (Tunis).

The first work of importance concerning the association of rickettsia and typhus was that of da Rocha-Lima published in two papers in 1916.

In the first paper (1916[1]), da Rocha-Lima mentions observations made with von Prowazek before his death in which rickettsia were found in great numbers in lice from typhus patients. It was during this work that von Prowazek and da Rocha-Lima became infected with typhus and von Prowazek died.

Da Rocha-Lima continued the work and established the frequent presence of the organisms in question in lice from typhus patients. He insisted that Giemsa's stain was necessary for their demonstration, since the ordinary stains used for bacteria stained them unsatisfactorily if at all. He described them as organisms with contours less sharply defined than those of bacteria and consisting of two substances, one staining faintly and the other, usually at the poles, taking a deep stain. The heavily stained polar portions are joined by the lightly staining outer substance. In the dark field these bodies looked to him like paired granules; in water they disintegrated. Measurements given by da Rocha-Lima were 0.3μ by 0.4μ for single granules, 0.3μ by 0.9μ for double granules or rods.

Da Rocha-Lima was the first to study these organisms in sections of lice and found them in seventeen or eighteen lice sectioned while none were found in a hundred normal lice. He discovered that these bodies multiplied within the epithelial cells of the louse's stomach and observed the changes produced in the cells by the growth of the organisms, distension and finally rupture.

In smear preparations he found similar bodies in three lice from non-typhus sources, a fact which he did not attempt to explain.

Later in the same year (1916[2]) da Rocha-Lima, in honor of Ricketts and Prowazek, gave the name *Rickettsia prowazeki*

to this micro-organism. He called attention to differences between rickettsia and bacteria. He discovered that a temperature above 23° C. was necessary for the development of rickettsia in lice and recommended 32° C. as the optimum temperature. A period of at least five days is required for the development in lice to reach a demonstrable stage.

Nöller (1916), in a few experiments with lice, confirmed da Rocha-Lima's observations in regard to the appearance of rickettsia in lice fed upon typhus patients.

Töpfer and Schüssler (1916) reported results confirming da Rocha-Lima's in regard to the occurrence and intracellular position of rickettsia in lice fed upon typhus patients. They did not find them in lice from healthy individuals or from patients with other diseases. For their material lice collected from typhus cases were used as well as lice experimentally fed upon patients. No infected lice were found before the fourth day while after the ninth day all were found infected. The illustrations and descriptions given correspond with those of da Rocha-Lima.

Töpfer (1916) later reported work based upon the examination of more than five thousand lice, examined mostly by means of smear preparations but also by sectioning. He did not agree with da Rocha-Lima in separating rickettsia from bacteria and regarded them as identical with the coccobacilli described by Sergent, Foley, and Vialette and maintained that he could not discover notable differences in their staining reactions as compared with bacteria. The type form of rickettsia he regarded as a bacillus and the other forms described, as stages in division and growth. Töpfer also could not confirm von Prowazek's conclusions in regard to the paired granules in neutrophilic leucocytes in typhus, as he also found indistinguishable appearances in leucocytes from other febrile conditions. He called attention to the fact that saprophytic bacteria could multiply within cells in the louse. However, Töpfer unreservedly concludes from his own investigations that this micro-organism is the cause of typhus, and in all

essential points agreed with the conclusions and observations of da Rocha-Lima. He was able to infect head lice with the organism but insisted upon calling it a bacterium.

Otto and Dietrich, in 1917, confirmed the presence of *Rickettsia prowazeki* in lice fed upon typhus patients. In their experiments of feeding 3000 lice upon 62 patients, 55 per cent of their lice died. Of the 45 per cent which survived the experiments, 20 per cent were infected with rickettsia. In 25 per cent rickettsia could not be demonstrated. In several experiments, where the lice were permitted to feed sufficiently long, 75 to 80 per cent became infected. The period of the disease when lice are most easily infected is between the fifth and the seventh days. After the thirteenth day the patients no longer infect lice.

Otto and Dietrich (1917, p. 579, fig. 2) described thread-like forms of rickettsia from recently infected lice in addition to the conventional rod and bi-polar forms and regarded these thread forms as precursors of the small forms.

Agglutination tests with suspensions of rickettsia obtained from lice while not wholly satisfactory were interpreted by Otto and Dietrich as confirmatory of the etiological relationship of rickettsia to typhus.

Other rickettsia in lice were found in 1916 by Töpfer (1916[1,2,3]) in association with Wolhynian fever or trench fever, and by Munk and da Rocha-Lima in 1917 in presumably normal lice. Töpfer (1916[1,2]) reported the finding, in the blood of trench fever patients, of diplo-bacillary and diplo-coccoid bodies, and claimed to have produced a febrile reaction in guinea-pigs by the inoculation of human trench fever blood and to have observed the same bodies in the blood of the guinea-pigs. In a third paper (1916[3]), he reported the presence of the organisms in lice fed upon Wolhynian fever patients. These results were substantiated by Jungmann and Kuczynski (1917[1,2]) who also found diplo-bacillary forms in the blood of patients and in the feces and intestinal tract of lice fed upon them. They were unable to differentiate between the micro-

organisms of typhus and Wolhynian fever and apparently did not study their lice by sectioning. They found 80 per cent of the lice collected from cases of the disease to be infected.

Munk and da Rocha-Lima (1917) found rickettsia in lice from Wolhynian fever cases, but could not prove their relationship to the disease, as they found them in lice fed upon cases of other diseases as well as upon normal people. They showed that these rickettsia in lice from various sources were different from the rickettsia associated with typhus in that they multiplied and remained extracellular in the louse's alimentary tract. They gave the name *Rickettsia pediculi* to this type of micro-organism and denied it pathogenic properties. They pointed out other less important differences which are described below. They criticized the finding of these bodies by Jungmann and Kuczynski in the blood of patients on the basis that similar paired granules can be found in Giemsa preparations of normal blood.

The fact that lice acquired *Rickettsia pediculi* when fed upon Wolhynian fever cases after recovery was used as further evidence against their etiological significance. Munk and da Rocha-Lima did not accept the transmission of Wolhynian fever by the louse as proved.

Meanwhile, Nöller's discovery (1917) of a rickettsia in the "ked" ("louse," "tick," *Melophagus ovinus*) of sheep and not associated with any ovine disease, furnished material for skepticism.

Brumpt in 1918, and Strong in 1919, opposed the conclusions that any rickettsia were pathogenic. Brumpt found rickettsia in lice from presumably healthy prisoners of war and did not experience any ill effects from nurturing these lice in numbers upon his own person. He did not distinguish between extracellular and intracellular varieties of rickettsia. Strong also fed lice containing rickettsia collected from healthy persons upon other healthy individuals without observing any ill effects.

Adequate control experiments with the objective of determining the specificity of rickettsia for any disease had not

been done, and apparently had not been seriously undertaken until the League of Red Cross Societies sent our Typhus Research Commission to Poland.

English workers, Arkwright, Bacot, and Duncan, and Byam and Lloyd in 1919, presented considerable casuistical evidence for rickettsia as the cause of trench fever.

A brief summary of our knowledge of rickettsia available at the time of writing, June, 1921, seems desirable and for that reason is presented in this report.

2. Summary of Present Knowledge of Rickettsia

A satisfactory definition of rickettsia is not possible at present. The properties in common of the thirteen or fourteen micro-organisms so far described under this name are as follows.

Morphology: Bacterium-like on the whole. They are smaller than bacteria and occur characteristically in pairs. Large forms, bacillary and filamentous, have been described in connection with two carefully studied rickettsia — *Rickettsia prowazeki* and *Rickettsia lectularius* — and it seems probable that a simple cycle or sequence in morphological development is a characteristic of the pathogenic forms.

Staining Reactions: Difficulty of staining with the common staining solutions used for bacteria is a striking feature, as is the failure to retain the stain by Gram's method. The only satisfactory staining methods are the modifications of Romanowsky's method; of these, the most satisfactory is Giemsa's solution.

Motility: Motile forms have not been seen.

Cultivation: So far all have resisted cultivation with the exception of the rickettsia from the sheep louse. It grows on a relatively simple glucose blood agar medium.

Resistance to Physical and Chemical Agents: Not enough work has been done to generalize. The viruses of typhus (da Rocha-Lima, 1919[2], p. 240) and Rocky Mountain spotted fever (Wolbach, 1919) are extremely susceptible to heat, dry-

ing, and chemical agents. On the other hand, the virus of trench fever resists 80° C. of dry heat for twenty minutes and drying for many months (Byam and Lloyd, 1919).

Host Specificity: All rickettsias have insect hosts which in the case of the pathogenic ones are the vectors. All are highly specific for their insect host while the pathogenic ones may infect widely separated mammals.

Hereditary Transmission: In every instance where careful study has been made it has been found — with the exception of the rickettsia of typhus — that the organisms pass down through successive generations, in the eggs. Da Rocha-Lima has offered some evidence that this is also true of *Rickettsia prowazeki*, and Sergent, Foley, and Vialette, 1914 (quoted by Nuttall, Parasitology, Vol. 10), accidentally communicated typhus to a monkey and a man with the offspring of lice which were supposed to be infected only with relapsing fever.

Classification: Is of course impossible, and it is probable that we have already included under rickettsia a number of very different micro-organisms. The *Rickettsia* of the sheep louse has little to distinguish it from *bacterium;* yet we believe the rickettsia of typhus has a number of peculiarities which necessitate its separation at present. The rickettsia-like cause of Rocky Mountain spotted fever, which we prefer for the present to consider under a distinctive name, while resembling in many ways *Rickettsia prowazeki*, is very unlike the morphologically simple rickettsia of trench fever.

The following table (Table X) indicates the wide distribution of "Rickettsia" in "insects." One important generalization, which we believe to be warranted in view of their great specificity for their insect hosts and hereditary transmission, is that they are forms of micro-organisms primarily adapted to insect tissues with occasional representatives pathogenic for mammals. It must be kept in mind that the grouping of these micro-organisms under *Rickettsia* can only be tentative with the meagre data so far determined.

TABLE X

Mallophaga...*Melophagus ovinus* (sheep "louse" or "tick") *Rickettsia melophagi*	Nöller	1917
Corrodentia...*Psocus Sp.?* (dust louse) Unnamed rickettsia	Sikora	1918
Insecta — *Hemiptera* — *Pediculus humanus* (human louse) *Rickettsia prowazeki*	Hegler and von Prowazek	1914
" (*rocha-lima?*)	da Rocha-Lima	1916
	Weigl, oral statement	1920
" *pediculi*	Munk and da Rocha-Lima	1917
" *quintana*	Munk and da Rocha-Lima	1917
	Schmincke	1917
" *wolhynica*	Toepfer	1916
Cimex (Acanthia) lectularius (bedbug) *Rickettsia lectularius*	Bacot	1921
Diptera ......*Culex pipiens* (mosquito, Europe) Unnamed rickettsia	Nöller, quoted by Sikora	1920
Siphonaptera — *Ctenocephalus felis* (cat flea) *Rickettsia ctenocephali*	Sikora	1918
Ctenopsylla musculi (mouse flea) Unnamed rickettsia	Sikora	1918
Dermacentor venustus (wood tick, United States) *Dermacentroxenus rickettsi*	Ricketts	1909
	Wolbach	1919
Arachnida Acarina — *Leptus (Trombidium) akamushi* (Harvest mite, Japan) Unverified quotation by Sikora		1920
Dermanyssus Sp. ? (Bird mite, Europe) Unnamed	Nöller, quoted by Sikora	1920

NOTE: Hindle (Parasitology, June 21, 1921, Vol. 13, No. 2, p. 152) has reported, without description, the existence of rickettsia in lice of the horse (*Trichodectes pilosus*) and of the goat (*Linognathus stenopsis*). To these new rickettsias he has given the names *Rickettsia trichodectae* and *Rickettsia linognathi* respectively.

The following data concerning the rickettsia in the preceding table are available.

Rickettsia melophagi: This micro-organism was discovered by Nöller in 1917 while studying flagellates of the sheep louse or tick. He succeeded in cultivating it upon a glucose blood agar medium. Jungmann in 1918 confirmed Nöller's work, including the cultivation, and determined that the infection of the insect host is hereditary. *Rickettsia melophagi* is not pathogenic, it occurs characteristically upon the cuticular surface of the epithelium of the sheep louse's stomach; but it may invade the cells. In morphology it corresponds to the small or coccoid forms of *Rickettsia pediculi* and *Rickettsia prowazeki;* it is not pleomorphic.

The unnamed rickettsia from the dust louse, *Psocus,* described by Sikora in 1918–20, is not associated with any mammalian host. It is transmitted hereditarily, lives wholly extracellular in the stomach of its host, and is non-pathogenic. Sikora was unable to infect *Pediculus* with this rickettsia.

Rickettsia pediculi, Rickettsia quintana, and *Rickettsia wolhynica* (Plate I, figs. 3 and 4) are morphologically indistinguishable and in all probability are identical (Wolbach and Todd, 1920), (Bacot, 1921). They are on the whole easier to stain than *Rickettsia prowazeki,* and are much more uniform in morphology. They are slightly plumper and more definitely oval than *Rickettsia prowazeki.* They occur characteristically extracellular in the louse's stomach and adhere to the cuticular border of the stomach epithelium in a striking manner (Plate V, fig. 20). Sikora and others maintain that exceptionally *Rickettsia pediculi* invades the epithelial cells of the louse's stomach. We have not been able to find evidence of intracellular distribution or multiplication of the rickettsia acquired by the stock lice fed upon Mr. Bacot during his illness with trench fever although twenty-six were examined by serial section. It is transmitted hereditarily in the louse. The virus of trench fever (and therefore *Rickettsia pediculi,* if we are correct in our conclusions) resists a dry heat of 80° C. for twenty minutes, and ordinary dessication in sunlight for

long periods (four months). This is in great contrast to the behavior of the viruses of typhus and of Rocky Mountain spotted fever.

Rickettsia prowazeki: Is strikingly pleomorphic, multiplies exclusively within cells in the louse and is very susceptible to drying and heat. Da Rocha-Lima has produced some evidence that in the louse it is transmitted hereditarily.

Rickettsia rocha-lima: Weigl in Warsaw in 1920 showed us preparations of lice containing intracellular pleomorphic rickettsi indistinguishable by us from *Rickettsia prowazeki* (Fig. 2, plate I). According to Weigl this is a non-pathogenic rickettsia infecting man, and was acquired by lice fed upon himself and upon members of Denekine's army. We are not convinced that Weigl was dealing with a rickettsia other than *Rickettsia prowazeki.*

Rickettsia lectularius: Arkwright, Atkins, and Bacot, 1921, is an extremely interesting micro-organism, morphologically very similar to *Rickettsia prowazeki* in pleomorphism and staining. It is non-pathogenic and is apparently widely distributed in bedbugs in England and Europe. (Fig. 19, plate IV, illustrates a smear, made by Mr. Bacot, of a bedbug caught in Warsaw.) It multiplies exclusively intracellularly in various organs of the bedbug and is transmitted hereditarily. Arkwright, Atkins, and Bacot describe a morphological cycle or sequence of forms, similar to that of *Rickettsia prowazeki* and *Dermacentroxenus rickettsi.*

The unnamed rickettsia from *Culex pipiens* discovered by Nöller and referred to by Sikora, 1920, occurs extracellularly in the mosquito. No further data are furnished.

Rickettsia ctenocephali: Sikora (1918), is a non-pathogenic micro-organism exhibiting large and small forms, but it is not as pleomorphic as *Rickettsia prowazeki* and *Rickettsia lectularius.* It occurs in the coelom of the cat flea and is transmitted hereditarily.

The unnamed rickettsia from the mouse flea was briefly mentioned by Sikora in 1918. It occurs intracellularly in the Malpighian tubules.

The unnamed rickettsia from the bird mite is another intracellular representative discovered by Nöller and mentioned by Sikora, 1920.

The rickettsia in the harvest mite is highly questionable. Sikora (1920) mentions it as a recently discovered cause of Tsutsugamushi disease, information which came by word of mouth.

Dermacentroxenus rickettsi (Plate I, fig. 6), the cause of Rocky Mountain spotted fever, has been included among rickettsia by a number of authors. It is therefore included in the above table. Nevertheless, comparison with *Rickettsia prowazeki*, while showing a number of common features, brings to light many differences between the organisms in morphology and in behavior in the insect vector.

Dermacentroxenus is less bacterium-like than any of the rickettsias and in multiplicative form always shows red and blue staining materials. It does not appear in thread-like or filamentous forms as does *Rickettsia prowazeki*.

In the louse, *Rickettsia prowazeki* continues to multiply indefinitely in the stomach epithelium; it eventually causes the death of the louse through suspension of digestion. *Dermacentroxenus*, however, after a stage of active multiplication, largely intranuclear, floods all the tissues of the tick and then diminishes in numbers, leaving behind in certain tissues including the salivary gland forms different from the multiplicative forms, and which Wolbach regards as a resistant stage.

For the present we prefer to keep separate dermacentroxenus — a parasite in *Arachnida* — from the rickettsia which are parasitic in *Insecta*.

Cross immunity experiments, now in progress, show that guinea-pigs which have recovered from typhus do not react as do normal guinea-pigs to inoculation with Rocky Mountain spotted fever. While typhus immune guinea-pigs develop spotted fever, they all show a lengthened incubation period and lower temperatures. The mortality was also strikingly reduced by about 50 per cent.

The rickettsia that concern us most are those in lice. The early control work by Brumpt and Strong, apparently based wholly upon the examination of smear preparations, showed the presence of rickettsia in lice from presumably disease free persons in France. Munk and da Rocha-Lima in 1917 showed that there were two types of rickettsia in lice, one extracellular, which multiplies wholly outside of the epithelial cells of the gut in the lumen and upon or in the cuticular border of the cells, and one associated with typhus which multiplies exclusively within the epithelial cells of the louse's gut. The former he named *Rickettsia pediculi* and he believed it to be a non-pathogenic micro-organism confined to the louse. The latter is *Rickettsia prowazeki*. In no control work has it been shown that intracellular rickettsia occur in lice from a certainly typhus free population, although both da Rocha-Lima and Toepfer state that very rarely *Rickettsia pediculi* may invade the cells. We, in view of the heavy typhus infestation of the countries in which this work was done, and our failure to find intracellular rickettsia in the controlled British stock lice fed upon Mr. Bacot during his illness with trench fever, regard these observations as open to strong doubt. Twenty-six lice of the British stock fed upon Mr. Bacot during his illness were studied in sections. Seventeen contained extracellular rickettsia. Nine were negative. In none did we find intracellular rickettsia. On the other hand, since the association of rickettsia with trench fever has been given etiological significance by Toepfer (1916), Munk and da Rocha-Lima (1917), Arkwright and Bacot (1919), Byam (1919), and since the rickettsia observed by these authors are of the extracellular proliferating type and indistinguishable from *Rickettsia pediculi* of da Rocha-Lima, the question of deciding the specificity of rickettsia for trench fever is a most difficult one. The query is: are *Rickettsia quintana (wolhynica)* and *Rickettsia pediculi* identical? If so, is this rickettsia the cause of trench fever?

4. Rickettsia and Trench Fever

Our control work with lice in Warsaw demonstrated the common (12 in 148) occurrence of exclusively extracellular rickettsia in lice collected at a public bath-house. Mr. Bacot, of our Commission who collected and worked with these lice between March 31st and April 5th, developed on April 7th a sharp febrile attack and subsequently underwent a course of illness corresponding objectively and subjectively with trench fever. He was carefully studied by ourselves and by Dr. Kruger of the American Red Cross. Mr. Bacot during this period was feeding upon his person the stock of lice brought from England, which were known to be free[1] from rickettsia for a period of over two years before our work and which were carefully controlled by us just prior to Mr. Bacot's illness.

On April 27th rickettsia began to appear in Mr. Bacot's stock lice. They shortly appeared in enormous numbers and study of the lice by serial sections showed that they were always extracellular. Now follows what we regard as the most important observation of all — that Mr. Bacot (Brit. Med. Journ., January 29, 1921, p. 156) continued to infect clean stock lice fed upon his person with rickettsia long after his recovery, as late as September, four months after the attack and three months after the disappearance of all symptoms. We have here strong presumptive evidence that Mr. Bacot acquired his infection from the Warsaw lice and that the rickettsia transmitted by him to his own stock of lice were identical with those in the Warsaw lice. We believe that these experiences constitute strong evidence for the identity of *Rickettsia pediculi* and *Rickettsia quintana* (or *wolhynica*) as well as for the etiological relationship of rickettsia to trench fever. This belief, of course, predicates that the mass of population in Central Europe is immune to trench fever, and tolerant of *Rickettsia pediculi* infection. For the present then we must assume that there is but one type of extracellular rickettsia in

[1] Compare Hindle, "Notes on *Rickettsia*," Parasitology, June, 1921, Vol. 13, No. 2, p. 153.

human lice (*Rickettsia pediculi, Rickettsia quintana, Rickettsia wolhynica*).

According to Strong's (1918) experiments the virus of trench fever is not transmitted by infective lice to their ova, while according to a number of observers, including Bacot, *Rickettsia pediculi* are found in the ova; a discrepancy which indicates that the virus of trench fever and rickettsia are separable. The positive filtration experiments of Strong with trench fever virus may also be regarded as evidence contrary to acceptance of rickettsia as the cause of trench fever.

RICKETTSIA PROWAZEKI IN LICE

THE gross appearances of lice removed from the experimental boxes was carefully observed in order to ascertain whatever characteristic might appear as indicative of a rickettsia infection. It was suggested (Weigl, 1920) that distended pinkish colored lice which appear in boxes after periods of prolonged feeding are most certainly ones infected with rickettsia. This we could not confirm as regards color. Lice which remained swollen after forty-eight hours fasting we found to be almost invariably heavily infected with *Rickettsia prowazeki*. In heavily infected lice digestion is suspended or greatly retarded owing to the loss in function of practically all of the cells of the mid gut due to the packing of their cytoplasm with the rickettsia. The swollen appearance after fasting proved to be the only reliable gross evidence of infection; many lice which were moribund, swollen, or discolored at the time of their removal proved not to be infected.

Epithelial cells swollen with *Rickettsia prowazeki* (Figs. 24 and 25, plate VII) were frequently seen in the louse's stomach during dissections under the binocular dissecting microscope (magnification of 22.5 diameters). They appear as pale whitish spherules against the dark brown background of intestinal contents, when obliquely illuminated by reflected light.

Staining: All our descriptions are based upon preparations stained with dilute Giemsa's stain (see section on technic, p. 12). We made no effort to devise other methods of staining. Our attempts to apply the usual stains employed for bacteria gave the results noted by previous investigators — failure. *Rickettsia prowazeki* does not retain the stain by Gram's method and will not stain with aqueous solutions of the dyes usually employed as counter stains, Bismarck brown and pyronin. They will not stain with aqueous solutions of

methylene blue and stain very feebly with Loeffler's blue applied with heat. According to da Rocha-Lima diluted carbol fuchsin and carbol thionin, as employed for bacteria, stain rickettsia feebly if at all, while concentrated solutions of carbol fuchsin and carbol gentian violet will stain them but not more heavily than bacteria can be stained with the dilute solutions.

With Giemsa's stain, used as described above, the small forms of *Rickettsia prowazeki* stain clear rose red, much less intensively than bacteria which (in the same preparation) stain deep purple or bluish red. On the whole the staining of rickettsia, in color and in intensity, is like that of spirochaetes. Certain larger thread-like or filamentous forms as well as the substance intermediate between the poles of small forms stain a light clear blue. (Figs. 8, 9, 11, etc., plate II.)

Rickettsia may also be demonstrated by Burri's method. Nigrosin in saturated aqueous solution is more satisfactory than India ink; while the use of an aqueous solution of cyanochin blue, which Weigl called to our attention, gives the combined advantage of a dark background and a faint tinting of the intermediate substance. While these modifications of Burri's method have contributed no new information in our study of *Rickettsia prowazeki*, they demonstrate that the differential staining, polar bodies, and intermediate substance, correspond with physical differences (Fig. 10, plate II).

Dark field illumination with preparations from heavily infected lice shows *Rickettsia prowazeki* as paired and single ovoid bodies without motility but exhibiting molecular motion to a degree shown by other particles of equal size. The dark field is reliable for the identification of *Rickettsia prowazeki* only when they are present in numbers. We employed dark field illumination on nineteen occasions for the examination of suspensions of louse organs, and recognized rickettsia in eleven instances in material which showed the organisms in stained preparations; but we failed to identify them on two occasions when stained preparations revealed them.

The refractivity of *Rickettsia prowazeki* seems to us to be less than that of bacteria and more like that of spirochaetes.

The double contour seen with most bacteria under dark field illumination does not show with *Rickettsia prowazeki*. Da Rocha-Lima states that rickettsia do not differ from bacteria in their power to refract light.

1. SMEAR PREPARATIONS OF LICE

The rickettsia of typhus literature is a small bacterium-like micro-organism, ovoid or elliptical in shape, usually occurring in pairs. (Figs. 12, 14, and 18, plates II, III, and IV.) Short and long rods with polar bodies and coccoid and bacillary bodies in short chains have been described (Fig. 13, plate III). The ovoid coccoid form in pairs is usually regarded as the type form. The two elements composing the pairs stain deeply with Giemsa's stain, taking a red coloration while the material between the two stains pale bluish. Where the organism occurs singly there is often a zone of pale blue material surrounding or tapering from the deeply staining red ovoid body. The minute paired forms, while composed usually of ovoid elements, are often composed of two pyriform elements joined at their narrow extremities lying in a straight line or forming an obtuse angle. These pyriform elements may be unequal in size. They stain blue and contain in their distal extremities red stained, round, ovoid, or ellipsoidal bodies. They seem to be a variation or possibly a divisional stage of the blue staining rod form with polar bodies described below. (Fig. 15, plate III.)

The minute coccoid and paired coccoid forms were invariably present in greater or less numbers in all our preparations from rickettsia infected lice.

Measurements by us from negatives of photomicrographs, made accurately at 2,000 diameters and read under low magnification with a micrometer ocular calibrated so as to make ten divisions of the scale equal one millimeter, give the following dimensions. The smallest single elements range from 0.25μ by 0.4μ to 0.3μ by 0.45μ. The paired forms range from 0.25μ by 0.7μ to 0.3μ by 1.1μ. (The above method of measuring micro-organisms is probably accurate to 0.05μ.)

These measurements correspond closely to those of da Rocha-Lima who gives 0.3μ by 0.4μ for single elements and 0.3μ by 0.9μ for the paired forms.

In all smears, in addition to the small forms described above we have noted the presence of small numbers of slightly larger more deeply staining and more uniformly shaped coccoid paired bodies, somewhat lanceolate in shape and devoid of bluish staining intermediate substance. These forms can be recognized in sections of lice and are the forms most easily demonstrable in human tissues.

In the systematic examination of one hundred and eighteen smears containing Rickettsia prowazeki, the rod-like forms mentioned by da Rocha-Lima with deeply stained polar granules (Fig. 12, plate II) were seen frequently in large numbers and almost invariably in small numbers. They are proportionately most numerous in sparsely infected lice and stain best when embedded in fragments of cytoplasm. The polar granules stain deep red, the body of the rod a light clear blue, their dimensions range from 0.25μ to 0.35μ in width, to 1μ to 2.5μ in length. Other palely staining rods, much swollen in the central portion and capped at each end with a biscuit-shaped red staining body, occur in the presence of the bacillary forms and probably represent degeneration or involution forms. A third form, which has been described previously only by Otto and Dietrich (1917), has been seen often in smears by us, and is associated with early infection of the louse or of individual cells in the louse. This is a filamentous form (Fig. 13, plate III) and its association with early infection of cells is based upon the study of sections of infected lice.

For a considerable period in our work we avoided decision upon delicate filamentous forms which appeared only in lice fed upon typhus patients and never in the control lice. Constant association of these forms with the minute forms in a single chain of organisms, in the same louse cell, in the same louse or in other lice from the same boxes with the failure to find them in smears from any louse which, though fed upon typhus cases, did not produce typhus when injected into a

guinea-pig, compelled us to accept them as a form of *Rickettsia prowazeki*.

These filamentous or thread-like forms in smear preparations are usually curved, sometimes sharply flexed in one or several places (Fig. 8, plate II). In width they range from 0.3μ to 0.4μ and in length are often extraordinary, 10μ to 40μ or 50μ (Fig. 9, plate II). The threads stain blue and the outlines seem unbroken though varying slightly in width in the same filament. Within the blue stained filaments are red stained bodies in pairs and chains corresponding in dimensions to the free lying rickettsia bodies. Some of the thread forms stain homogeneously — pale blue. Within a single filament (Fig. 8, plate II) the arrangement of the red stained bodies suggests the division of the whole into any one of the previously described forms, coccoid bodies, the thick or slender bi-polar bacillary, and all forms, singly, in pairs, and in long or short chains. In occasional preparations we can identify portions of crushed epithelial cells from the louse gut filled with enormous numbers of filamentous, rod, and coccoid forms with apparent stages in the formation of the two latter forms from the former (Figs. 13 and 29, plates III and IX).

The early occurrence of these filamentous forms is borne out by finding them in sections of lice lying curled within non-swollen cells of the mid gut at a time when very few cells are infected with the coccoid rickettsia. Similar bacillary and thread-like or filamentous forms have been described by Arkwright, Atkins, and Bacot as a part of the developmental cycle of a rickettsia-like (*Rickettsia lectularius*) parasite of the bedbug (1921). These authors suggest that the small *Rickettsia lectularius* bodies develop through the bacillary stage into filaments while others continue to multiply by simple fission. The filamentous forms finally break up into the minute rickettsia bodies. An intracellular development of minute forms also leads to the formation of larger lanceolate paired forms from which bacillary and filamentous forms develop.

2. Rickettsia Prowazeki in Sections of Lice

Sixty-two of the lice sectioned from the experimental boxes contained *Rickettsia prowazeki*. Seven of these also contained extracellular rickettsia which, because of their position upon the cuticular borders of the cells, deep staining and uniform morphology, we decided to call *Rickettsia pediculi*. The staining technic employed by us stains *Rickettsia prowazeki* in sections blue or purplish blue. *Rickettsia pediculi* stain purple red or red.

In a few lice the only evidence of rickettsia was the presence of one or a few non-swollen cells filled with bacillary to thread-like forms. A larger number contained only a few cells filled with bacillary forms (Fig. 28, plate IX) and these cells were sometimes not swollen or more often swollen and even appeared as pedunculated projections into the lumen of the gut (Fig. 30, plate IX).

In all lice where a large number of cells were infected, the minute coccoid form predominated, and cells containing these are usually swollen to many times their normal size and are easily recognizable with low power objectives (Figs. 24 and 25, plate VII). The multiplication of these coccoid forms leads finally to rupture of the cells. Two types of small diplococcoid bodies can be recognized within cells in sections, and these correspond to forms seen in smears; one staining blue or bluish, the smallest recognizable form of rickettsia, and the other slightly larger, lanceolate and more deeply stained (Figs. 26 and 31, plates VIII and IX). If the differentiation of the stains is carried far enough or if the slides after completion are bleached in sunlight these more deeply stained pairs retain a purple red color and seem always to be within vacuoles.

In the lightest, and therefore probably the earliest, infections observed in lice, the organisms have been bacilliform. The rickettsia have always been bacilliform in lice where prolonged search has resulted in the discovery of a single infected cell. From our observations it is not possible to state whether

infection commences with the rod-shaped rickettsia or with some other form — possibly the minute paired granules which have been observed in swollen cells of lice, certainly exposed to infection, in which definite rickettsia are not found (Box LII). The bacilliform stage when present in large numbers (Fig. 30, plate IX) distend the cell and the rod elements seem to grow in chains which are parallelly arranged in coils within the cell. When present in small numbers the cells containing them remain normal in appearance and only careful study with the oil immersion objective will reveal them, often in short chains irregularly arranged in various directions in the cytoplasm of the cell (Fig. 28, plate IX). Occasionally but one or a few rods are found in a cell, and these seem to be surrounded by a halo or unstained area.

In practically all lice containing the minute coccoid forms, the rod forms have been seen (Fig. 33, plate X).

The forms seen free in the lumen of the gut have been the small coccoid and pair forms, singly or in chains, and the thread or filamentous forms. The latter sometimes seem to gain entrance independently of rupture of the epithelial cells while the former probably only gain entrance into the lumen when the over-distended cells containing them rupture.

We have never been able to find rickettsia in any organ of the louse other than the alimentary tract. Our material suggests that the infection begins in the anterior part of the mid gut, probably in or near the diverticulae, and that the thread-like and bacillary forms precede the appearance of the small coccoid and streptococcoid forms.

We could not confirm Sikora's (1920) finding of rickettsia in the salivary glands. It is easy to exclude their presence from the tubular salivary glands. The reniform salivary gland cells are so filled with secretory granules that recognition of micro-organisms as minute as rickettsia is a matter of doubt. Comparison of salivary glands in sections of infected and control lice shows no differences and we believe that this organ does not become infected and that it cannot be a usual site of multiplication of *Rickettsia prowazeki*.

Rickettsia were never found by us free in the body cavity (coelom) of the louse, a noteworthy fact because during digestion pseudopodia project from the epithelial cells of the gut for a distance equal to half the length of the cell into the body cavity.

The feces from fourteen of the experimental louse boxes were examined for rickettsia. The organisms were found in the feces from four boxes only which contained many lice heavily infected with *Rickettsia prowazeki*. Infection of the feces was expected since the examination of louse sections had constantly revealed the presence of rickettsia free in the gut lumen. A series of twelve guinea-pigs was inoculated with feces known to be heavily infected with *Rickettsia prowazeki*. From temperature reactions it is believed that in five instances the animals inoculated contracted typhus; the presumption of typhus, in these experiments, is based solely on the temperature reactions and is not confirmed by histological examination of tissues.

Da Rocha-Lima (1919, p. 242) did not succeed in infecting guinea-pigs in two experiments with louse feces which had been dried for twenty-four hours at room temperature (22° C.). On the other hand Nicolle, Blanc, and Conseil (1914) did infect guinea-pigs with louse feces. In our experiments we did not intentionally dry the louse feces which were used for the injection of guinea-pigs and it is probable that some of the material was not thoroughly dry, although the lice had not been fed within twenty-four hours.

Ova, larvae, and nymphs from eight boxes which contained lice infected with *Rickettsia prowazeki* were examined in smear preparations. Rickettsia were found in no instances.

Curious, long, thick, densely staining red filamentous bodies (Fig. 16, plate III) were seen in the gut cells of four rickettsia infected lice from two experimental boxes. They exhibit no internal structure and some of them terminate in bulbous extremities. They are illustrated in Figs 7a and 7b, plate II. They are of varying diameters and of considerable length, so that it is necessary to follow them through several successive

serial sections to determine their shapes and sizes. Some are tapered and some contain clear spaces. They seem to be parasitic in nature. In some sections of experimental lice the rickettsia stain a brilliant red. In such preparations the chains of bacilliform rickettsia bodies may resemble the smaller specimens of the filamentous bodies. Indeed, in sections where both "filaments" and chains of bacilliform rickettsia occur it may be impossible to tell whether rickettsia chains or "filaments" lie in the field.

During prolonged search for rickettsia in sections of negative lice, bright red stained granules 0.5μ to 1.0μ in diameter have been found singly and in pairs in epithelial cells of the gut. These granules are quite distinct from precipitated stain. Their nature is wholly undetermined. In one louse, collections of uniformly red staining granules were seen in the fat body (see louse Box xxxviii). These collections measured about 20μ by 30μ; and seemed to have no definite limiting membrane. The granules which they contained varied in size but were usually about 2μ to 3μ in diameter.

In poorly fixed lice, where the fixative has not had free access to the organs owing to insufficient incision of the abdomen, the rickettsia in many greatly swollen cells appear as poorly-stained, poorly-defined granules and their presence can be confirmed only because of the great distension of the cell and the presence of unmistakable rickettsia in other parts of the same louse. In some experimentally fed lice upon typhus patients which we have recorded as negative moderately swollen cells with granular cytoplasm may actually have contained rickettsia.

It is also possible that a small proportion of the lice noted as uninfected in Table VIII harbored small numbers of rickettsia. Occasionally (Box xlviii, louse 18) an isolated cell packed with rickettsia in its bacillary form was found. A casual search of ten or fifteen minutes would usually fail to detect such a slight infection; and were a smear made of such a gut, the infection would almost certainly escape notice (Box lv, louse 4).

The higher percentage (28.3 per cent) of positive smears as compared with 21.5 per cent positive sections obtained by the examination of 705 lice from experimental boxes indicates that it is rather more difficult to find *Rickettsia prowazeki* by means of sections than by smears.

As a result of the combined study of smears and sections from infected lice, we believe that *Rickettsia prowazeki* undergoes a developmental cycle in the louse in which the first stages are the thread or filamentous and bacillary forms. The minute paired rickettsia bodies or coccoid forms probably represent the stage of most active multiplication. The deep stained lanceolate form is possibly the latest stage of development in the louse and a quiescent stage of slightly greater resistance to external agents.

3. THE RECOGNITION OF RICKETTSIA

In smears of lice and of tissues occur granules consisting of cellular débris and occasionally bacteria which cannot be definitely distinguished from isolated rickettsia. When rickettsia do occur in lice they are usually present in very large numbers, consequently, in practice rickettsia have never been definitely accepted as being present unless they occurred in considerable numbers and unless there was a wide range of variation in their size and form. Because of their pleomorphism, and because of uncharacteristic shapes, it is impossible to definitely identify any isolated organisms, or even group of granules, as rickettsia. In some of the Polish control lice, granules were seen which might have been rickettsia but which it was quite impossible to identify as such.

Because it is usually impossible to decide the origin of isolated structures seen in smears, the study of smears is often inconclusive and is much less satisfactory than the examination of sections. Among the heterogeneous and confusing débris seen in smears are hemoglobin crystals from the gut of lice. Sometimes these occur in very small rods and, if they take the stain, much resemble bacteria. Confusion is sometimes caused by granular substances representing coagulated and

half-digested blood contents. Occasional passing confusion may be caused by the presence of particles of spermatozoa in every stage of development from the testes of male lice. Another source of possible error in the examination of smears of rickettsia is the introduction of organisms by the fluids, such as stain or water used in the process of staining.

In sections, the cytoplasm of swollen epithelial gut cells may be granular and assume forms so like rickettsia that it is difficult to be certain that organisms are not present. The granular appearance of cells of the salivary glands and of Malpighian tubules especially may be confusing. The free border of the epithelial gut cells is covered with a cuticular layer. In sections it usually appears as a hyaline and structureless border. Sometimes, especially at the anterior end of the mid gut, the cuticular substance is disposed so as to produce a ciliated appearance. A tangential section of such an area may produce an appearance somewhat resembling a collection of cocci. *Rickettsia pediculi* are always on this border and fill its edgings deep between the epithelial gut cells.

X

TYPHUS IN GUINEA-PIGS

Owing to a paratyphoid infection, we lost seventeen of twenty monkeys sent to Warsaw from England, an experience which led us to restrict our animal experimentation to guinea-pigs. Three monkeys were inoculated with blood from two typhus patients. One Rhesus inoculated intraperitoneally with 20 c.c. of blood from patient 116 (sixth day of the disease) failed to react during an observation period of forty-four days. A *Cercopithicus* (*sabra* ?) inoculated intraperitoneally at the same time with 20 c.c. of blood from patient 116 reacted on the thirteenth day and its temperature remained high for six days.

Three guinea-pigs were inoculated from this monkey on the fifth day of temperature, each with 2 c.c. of blood given intraperitoneally. One, No. 63, died following a rise of temperature on the fourtheenth day, and the autopsy showed acute peritonitis. Two, Nos. 61 and 62, reacted in a manner typical of typhus, both on the fourteenth day. They were killed on the sixth day of temperature and the autopsies showed conditions consistent with typhus. The histological examination of guinea-pig 61 showed the typical lesions of typhus in the cerebrum and cerebellum and in blood vessels of the testes and appendages.

A third monkey, a *Cercopithicus* (*sabra* ?) inoculated intraperitoneally with 20 c.c. of blood from patient 149 (ninth day of the disease), showed an elevation of temperature from the tenth to the thirteenth days inclusive after inoculation. No controls were made upon this monkey.

The resistance of *Macacus rhesus* has been commented upon by Nicolle and Conor (1912). The apparent marked susceptibility of *Cercopithicus* is of interest as it has not been mentioned among the species tried for susceptibility to typhus.

The susceptibility of the guinea-pig to typhus was first shown by Nicolle, Comte, and Conseil in 1909, and soon after-

wards confirmed by Ricketts and Wilder (1910), Gaviño and Gerard (1910[2,3]), Anderson and Goldberger (1912). Nicolle (1917) showed that typhus could be maintained indefinitely in the guinea-pig by successive passages and that on the whole guinea-pigs were more reliable animals for this purpose than the lower monkeys.

Examples of the transmission of typhus to guinea-pigs by the injection of louse viscera and by human blood are given in the protocols (p. 53) of the experiments done to ascertain the specificity of *Rickettsia prowazeki* for typhus infected lice.

Our experiences in maintaining two strains of the virus for a period of over a year in guinea-pigs are of sufficient value to warrant brief presentation, together with the results of louse and human blood injections. The records of these strains, patient 116 strain started by the injection of blood from a patient, and Louse Box xviii strain, started by the injection of the viscera of louse 224, are shown in Charts 1 and 2.

1. METHODS OF INOCULATION

Whole blood drawn from the aseptically exposed heart of a deeply anaesthetized guinea-pig was invariably used in the inoculations from guinea-pig to guinea-pig. The syringe used was rinsed with a thirty per cent sterilized solution of sodium citrate and thoroughly emptied; a method which insures against clotting of the blood during the one or two minutes required for the procedure of drawing the blood and the injection of several guinea-pigs.

The heart of the donor was exposed, after removing the skin over the chest and abdomen, by cutting a window in the chest wall with red hot scissors.

Intraperitoneal inoculations were employed in the maintenance of the two strains which have been carried to the present time, one from patient 116 (guinea-pig 5) since May 13, 1920, Chart 1, and one from Louse Box xviii (guinea-pig 23) since May 15, 1920, Chart 2.
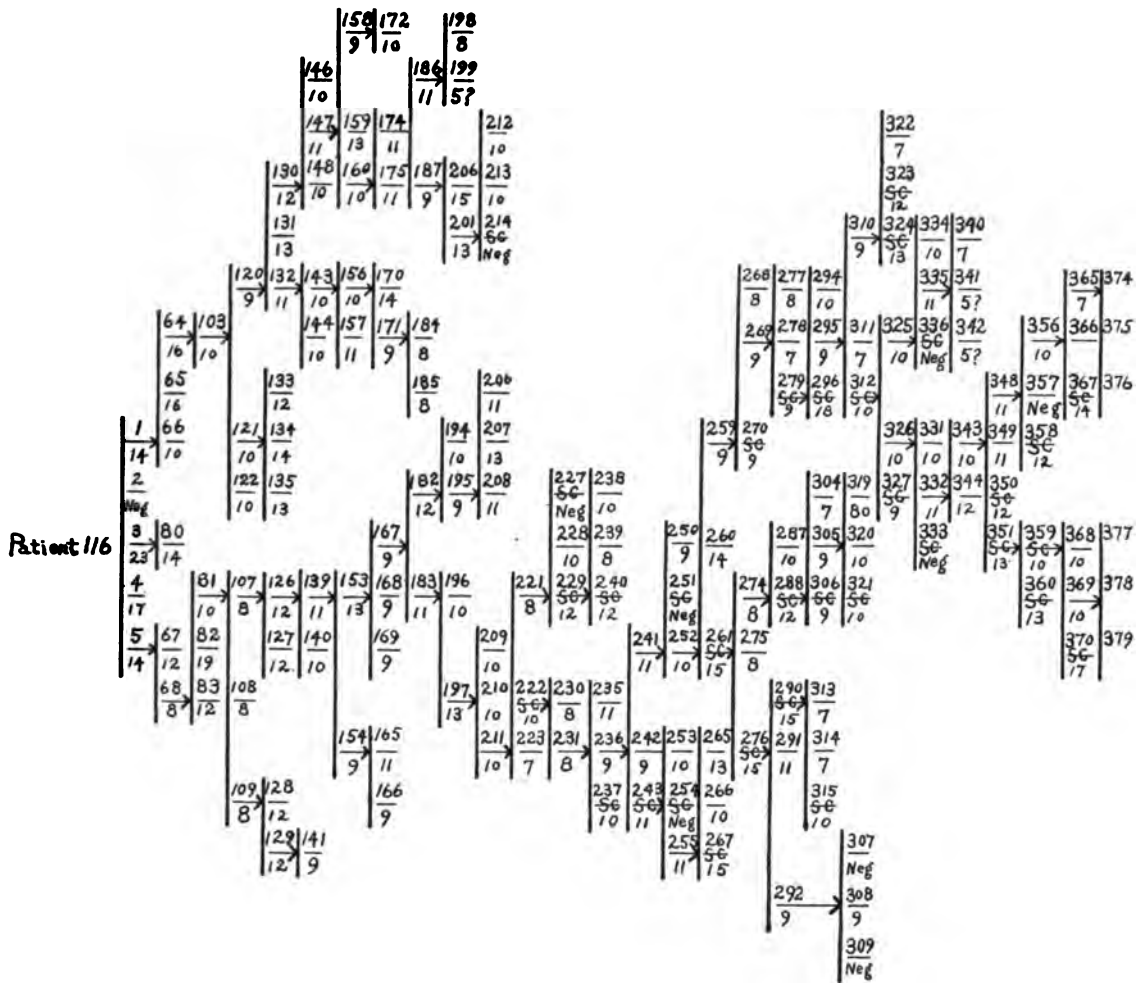
CHART 1. — Record of patient 116 strain of typhus in guinea-pigs, May 1920 to June 1921.
Guinea-pigs 1, 3 and 5 each received 10 c.c. of blood intraperitoneally on May 13, 1920. They ran normal temperatures until the 17th, 23d and 14th days respectively. Guinea-pigs 1 and 5 were killed on the fifth day of fever. The lesions of typhus were found in their brains. Guinea-pig 3 was killed on the seventh day of fever. No histological examination was made

The upper figure of each entry is the number of the guinea-pig, the figure below the line is the incubation period in days. S.C. indicates subcutaneous inoculation

CHART 2. — Record of Louse Box 18 strain of typhus in guinea-pigs May 1920 to June 1921. This strain was started from Louse 224.

See page 59 for record of Box 18, and page 61 for protocol of Guinea-pig 23

The upper figure of each entry is the number of the guinea-pig, the figure below the line is the incubation period in days.

S.C. indicates subcutaneous inoculation

Subcutaneous inoculations were employed from time to time as a means of eliminating mild secondary infections of the peritoneum which frequently appeared. Repeated cultures made from the heart's blood of guinea-pigs with a mild peritonitis in the early part of our work remained invariably sterile. In one instance a micrococcus was obtained by direct culture from the peritoneal cavity.

2. CLINICAL COURSE OF TYPHUS IN GUINEA-PIGS

The febrile reaction, discoverable only with the thermometer, is the only sign of the typhus reaction in the vast majority of instances. In our experience the guinea-pigs remain apparently well, continue to eat, and behave as normal animals. Pregnant females do not abort. The symptoms described by Anderson (1914), listlessness and loss of weight, in our experience have been due to the severer reactions due to secondary infections. Death from typhus in guinea-pigs has occurred very rarely. We have failed to make a careful study of the occasional deaths in our passage animals, but we believe that death occurring during the febrile period is always associated with secondary infections. The rare deaths (three cases) which we believe to have been due to uncomplicated typhus have occurred after one or more days of normal temperature during which the guinea-pigs have been paralyzed. The paralysis is spastic and resembles that of rabies. This behavior of typhus in guinea-pigs deserves more study. A typical example is that of guinea-pig 219 which was inoculated October 11, 1920 (Box xviii). (See Chart 2.)

Temperatures following inoculation:

	°F.			°F.
Oct. 11	102.4		Oct. 20	102.6
" 12	102.5		" 21	103.8
" 13	102.2		" 22	104.0
" 14	102.5		" 23	104.5
" 15	102.6		" 24	No record
" 16	102.6		" 25	104.4
" 17	No record		" 26	103.1
" 18	101.7		" 27	103.2
" 19	102.6		" 28	100.0

On the morning of October 28th the guinea-pig was found lying upon its side, spastic, making incoördinated attempts to arise. Its fur was in good condition, its eyes bright, but it did not attempt to eat. It remained in this condition throughout the day. It responded to handling by violent muscular efforts. After etherization that evening the skeletal muscles remained irritable long after the post mortem was completed — a phenomenon which we have seen frequently in rabid animals. The post mortem showed a moderately enlarged spleen, but no other definite lesions. The peritoneal surfaces were normal. The stomach and intestines were empty. The lungs, pleura, heart, and pericardium were normal. The brain showed a marked injection of the superficial blood vessels. The histological examination showed very extensive proliferative lesions throughout the cerebrum and cerebellum, identical with but much more numerous than those usually seen in typhus guinea-pigs.

3. Gross Pathology in Guinea-pigs

The changes to be noted are slight. The skin is negative. The inguinal and axillary lymph nodes are slightly enlarged, never deeply injected, usually pale or at the most pink in color. The peritoneal surfaces are normal. The spleen is usually slightly to moderately enlarged and deeper red in color than normal. The adrenal glands are occasionally moderately engorged with blood. In male guinea-pigs a noticeable injection of the testes and occasionally a small hemorrhage into the polar fat has been seen. The scrotal tissues and tunica remain normal and the reaction in our experience has been in no way comparable to that of Rocky Mountain spotted fever in guinea-pigs or as described with Mexican typhus by M. H. Neill (1917).

The brain is occasionally noticeably injected, but exhibits no positive gross evidences of the lesions found microscopically. The organs of the chest, liver, kidneys, and gastro-intestinal tract show no lesions or alterations in appearance from the normal.

The presence of a fibrinous exudate upon the spleen and omentum has often been found in guinea-pigs which exhibited no evidences of secondary infection during life, i.e., no shortening of the incubation period or unusual height of temperature. The inoculation of blood from such an animal into the peritoneal cavity of other guinea-pigs was usually, but not invariably, followed by a similar exudate while a subcutaneous inoculation was usually but not invariably followed by a normal peritoneum at autopsy. This appearance we feel certain is due to a secondary infection. In many guinea-pigs with moderately enlarged spleen we have found the spleen to be covered with a very thin transparent layer of fibrin-like material with normal peritoneum elsewhere. Smears as well as sections from such spleens show that this layer is composed of mononuclear cells and fibroblasts, without polymorphonuclear leucocytes and without demonstrable micro-organisms, and we believe that this delicate coating limited to the spleen is compatible with an uncomplicated typhus infection.

Nicolle and his associates unfortunately have given no detailed description of the pathology of typhus in guinea-pigs. They merely state that pathological changes are absent.

Otto and Dietrich (1918) note the following gross changes: swelling of the axillary and inguinal lymph nodes, slight swelling and reddening of the adrenal glands and minute hemorrhagic infarcts of the lungs and liver. They also state that the muscles may appear dry and friable and that the spleen is usually not notably enlarged.

Plotz, Olitsky, and Baehr (1915) note enlargement of the spleen.

Loewy (1916) has described macroscopically visible lesions on the under surface of the skin in typhus guinea-pigs. This we have not seen ourselves, and Otto and Dietrich (1918) also failed to find such skin lesions.

4. Incubation and Duration of Fever in Guinea-pigs

In judging of the incubation period and the duration of the febrile reaction it is necessary to recognize the effects of secondary infections. The results of many post mortems have shown that unduly short incubation periods, as well as a prolonged period of temperature, is due to the presence of secondary infection. As a rule a very short incubation period is followed by a prolonged period of fever, and the presence of these two signs may be accepted as proof of a complicating infection.

The incubation period of typhus in the guinea-pig rapidly becomes fixed within a definite period of days and does not become changed with long continued passages. The same is true of the duration of fever. Hence it may be said that the virulence is not reduced or augmented by repeated passages.

We have often noted a preliminary rise of temperature at the end of the incubation period, lasting but one day and followed by a remission of one day, then the period of constant fever as described by Nicolle.

The first passage from man to guinea-pig and from louse to guinea-pig may be attended by exceedingly long incubation periods, with the former, and exceedingly short periods with the latter. Forty days, according to Nicolle (1920) is the limit of incubation when injections are made from man to guinea-pig. According to da Rocha-Lima (1919) the injection of louse viscera may be followed by incubation periods as short as two days. Our own experiences do not corroborate these statements.

The following table (Table XI) gives the incubation periods in fourteen guinea-pigs which were proved to have acquired typhus through the injection of viscera of lice fed upon typhus patients.

With one exception, that of guinea-pig 9, inoculated with louse 227 which was heavily infected with rickettsia, no guinea-pig with uncomplicated typhus developed a reaction under seven days. The incubation period of four days in guinea-pig 9

TABLE XI

Guinea-pig	Louse	Incubation	Remarks
8	W 214, Box XIX	2 days	Autopsy two months later showed peritoneal adhesions and old pseudo-tuberculosis
48	W 4, Box XXXVIII	3 "	Secondary infection as shown by course of temperature
19	W 239, Box XIX	4 "	
22	W 241, Box XVII	4 "	Autopsy six weeks later showed active pseudo-tuberculosis
9	W 227, Box XVIII	4 "	No evidence of secondary infection
24	W 223, Box XVIII	5 "	Pseudo-tuberculosis, pneumonia
14	W 213, Box XIX	7 "	No evidence of secondary infection
15	W 228, Box XVIII	8–10 "	" " " " "
20	W 238, Box XVIII	9 "	" " " " "
47	W 3, Box XXXVIII	10–13 "	" " " " "
46	W 2, Box XXXVIII	13 "	" " " " "
18	W 215, Box XIX	13 "	" " " " "
23	W 224, Box XVIII	11 "	" " " " "
13	W 211, Box XIX	15–16 "	" " " " "

may have been due, as da Rocha-Lima believes, to a massive infection from the louse. The record of this guinea-pig is shown on page 65, under "Experiments to prove the Specificity of *Rickettsia prowazeki* for Typhus."

The incubation periods following intraperitoneal injections of blood from man (five patients) to guinea-pig (28 animals) were as follows (Table XII):

TABLE XII

Incubation	Number of Guinea-pigs	Incubation	Number of Guinea-pigs
9 days	2	16 days	5
10 "	1	17 "	3
11 "	1	18 "	1
12 "	2	19 "	1
13 "	1	23 "	2
14 "	3	25 "	1
15 "	5		28

The incubation periods following inoculations from guinea-pig to guinea-pig (passage virus) are shown in the following table (Table XIII):

TABLE XIII

Days	Number of Guinea-pigs	
	Subcutaneous Inoculation	Intraperitoneal Inoculation
4	0	1 ⎫ Secondary infection
5	0	2 ⎬ suspected because of
6	0	0 high and prolonged
7	1	13 temperature
8	1	26
9	4	40
10	6	58
11	1	29
12	8	12
13	6	12
14	2	5
15	3	3
16	0	2
17	2	0
18	1	0
19	0	1
	35	204

The three instances of incubation periods of less than seven days, since they occurred only in guinea-pigs inoculated intraperitoneally and were followed by an unusually long course of fever with unusually high temperatures, may safely be regarded as occasioned by the presence of a complicating infection.

Our conclusions in regard to the incubation period of typhus in guinea-pigs irrespective of the source of virus, i.e., from the louse, man or passage virus, are:

1. That the minimum incubation period of the uncontaminated virus is seven days.

2. That the repeated passage through guinea-pigs influences (shortens) the incubation slightly as compared with the first incubation period when inoculations are made from man to guinea-pigs, but has no further effect after the first few transfers.

3. That the 92 per cent of the guinea-pigs inoculated by us in maintaining the virus reacted between the seventh to thirteenth days inclusive.

4. That subcutaneous inoculations prolong slightly the incubation period.

These results practically coincide with those published by Nicolle (1912, 1917) and Anderson (1914).

The duration of the febrile period in guinea-pigs in our experience has not been influenced by repeated passages. Owing to the fact that most of the guinea-pigs inoculated were killed during the reaction period, we have data on only fifty-nine which in our judgment ran uncomplicated typhus courses. These are shown in the following table (Table XIV).

TABLE XIV

Duration of Fever	Number of Guinea-pigs	Duration of Fever	Number of Guinea-pigs
3 days	1	7 days	13
4 "	7	8 "	11
5 "	10	9 "	6
6 "	11	Total	59

Nicolle states that the period of fever lasts from four to eleven days. Anderson gives the extremes as four and eighteen days. From our experience a prolonged period of fever denotes secondary infections, particularly of the pseudo-tuberculosis type.

Nicolle, Anderson, da Rocha-Lima and others have noted apparent instances of guinea-pigs naturally immune to typhus infection. But Nicolle and Lebailly (1919) have pointed out that the virus of typhus may be present after the usual incubation period, without giving rise to fever in guinea-pigs, consequently absence of temperature is not proof of absence of infection.

Our own results are as follows:

In guinea-pig to guinea-pig inoculations, when done intraperitoneally, only seven of two hundred and four failed to react. In the case of subcutaneous inoculations, fourteen out of fifty failed to react.

In inoculations from man to guinea-pig, six failed to react of a total of thirty-four. One of these six was immune to a subsequent inoculation.

XI

PATHOLOGY OF TYPHUS IN MAN

1. Material

Thirty-nine post mortems were made upon the St. Stanislaus Hospital cases. One proved to be a case of general miliary tuberculosis and one case was that of a youth who died of pericarditis several weeks after recovery from typhus. The value of the remaining thirty-seven post mortems is largely due to the rapidity with which we were permitted to examine the bodies after death. Sixteen post mortems were made within two hours or less after death, seven were made between two and three hours. Only ten post mortems were done later than five hours. We are therefore able to base our descriptions upon tissues affected to a minimum degree by post-mortem changes, — an absolute essential for the highest type of histological work and for the demonstration of minute parasites in lesions.

The histology of the skin is further supported by twenty-eight specimens, excised under local anaesthesia during life, from cases in all stages of the disease.

The duration of the cases autopsied extended from the seventh day to the twenty-fourth (see Table XV) so that the series afforded ample material for the study of the evolution of the lesions in typhus.

2. Macroscopic Pathology

(a) *The skin:* Every case showed evidence of the rash, in the late cases small brownish areas only persisted in the sites of the previous lesions. The length of the post-mortem period affects the appearance of the rash in early cases as the macules fade rapidly after death. Petechial and larger areas of hemorrhage persist. Small petechiae and small thrombosed blood vessels in the skin may be demonstrated at post mortem by

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viewing reflected portions of the skin by transmitted light. Hemorrhages of considerable size were frequently found in the subcutaneous fat in sites subjected to slight trauma, e.g., hypodermic injections and bony prominences. Large areas of necrosis of the skin (gangrene), whether or not symmetrically distributed, were not accompanied by thrombosis of large vessels. Microscopical study of the skin necroses has shown that the non-symmetrical necroses are due to thrombosis of capillaries and small arteries and veins beginning in the corium and extending centripetally. This process may result only in small firm elevated yellowish red areas reminiscent of cutaneous gummata or in deep sloughs involving the whole depth of the subcutaneous fat and even the muscles (Autopsy No. 36) (Fig. 9). Stasis due to pressure apparently favors the localization of the endangeitis characteristic of typhus. The symmetrical gangrene of the extremities is probably due to nerve lesions; we had but one case of this sort at post mortem (Autopsy No. 26).

(b) *The organs of circulation and blood formation:* The blood is commonly stated to be dark and slow to coagulate. In general this proved to be the rule in our series. Clots in the heart were almost invariably small and soft and frequently the heart's blood was without clot as late as three hours post mortem; but, on escaping into the pleural cavity it formed a fairly firm coagulum. We noted no change in the viscosity of the blood.

(c) *The heart:* As a rule the left ventricle was contracted, and the right moderately dilated. The auricles, and particularly the left, were usually distended with blood. The myocardium in six cases which died, without important bacterial complications, between the ninth and twelfth days of the disease, showed pallor, loss of consistency, and yellowish streaks and points. These six post mortems were done soon after death and post-mortem changes were excluded. In a few other cases with confluent bronchopneumonia the same condition of the myocardium was found. The interpretation of the gross appearance of these hearts was substantiated in

each instance by the discovery of important histological lesions specific of typhus.

In the majority of the cases the myocardium showed no gross change beyond minute pale areas and lines evident only upon close inspection and microscopically; the hearts from all cases which died before convalescence showed some lesions. Acute lesions of the heart valves and macroscopic mural thrombi of the heart were not found.

(d) *Blood vessels:* The histological study of our cases shows that the large blood vessels occasionally are the seat of acute lesions specific of typhus. Such lesions in all probability are the foci upon which extensive thromboses form. We found one case of each of the following: Thrombosis of the superior mesenteric artery with infarction of the small intestine; thrombosis of the left internal carotid artery with massive cerebral softening of the left hemisphere; thrombosis of a main branch of the left pulmonary artery; thrombosis of the main branches of the splenic artery with infarctions; mural thrombi of the aorta and common iliac arteries.

(e) *The spleen:* The spleen of cases which died before the end of the second week, in most instances, was enlarged. The consistency was firmer than in typhoid and the cut surfaces remained smooth. The color of freshly cut surfaces was dark red or maroon-colored. In the third week the spleens were usually within the normal range of weight, even in the presence of severe or confluent bronchopneumonia. Table XV gives the weights of the spleens from our series of autopsies with the ages of patients, day of disease, and important complications which might affect the weight of the spleen.

(f) *Lymph nodes:* In cases uncomplicated by important infections, no gross changes were found in the lymph nodes.

(g) *Bone marrow:* Marrow from the middle third of the femur was taken from each case. Thirty-six of the thirty-eight typhus cases showed some degree of reaction, varying from a red mottling of the outer zone to a firm red marrow of non-fatty consistency. Histologically, the red portions of the marrow are actively erythroblastic. This reaction of the

TABLE XV. TABLE SHOWING DURATION OF THE DISEASE, HOURS POST MORTEM AND WEIGHT OF SPLEENS OF CASES AUTOPSIED

Autopsy No.	Hours Post Mortem	Duration of Disease — Days	Weight of Spleen Grams	Age and Sex	Important Complications
8	1	7	325	42 ♀	None
19	11	7	160	53 ♀	None
35	9	8	300	39 ♀	Confluent bronchopneumonia
3	7	9	230	Middle-aged ♂	None
7	4	9	440	53 ♀	None
9	2	9	325	37 ♂	Bronchopneumonia
1	7½	10	270	Middle-aged ♀	Bronchopneumonia
22	10	10	180	34 ♂	None
37	2¾	10	350	59 ♀	None
14	2	10	200	52 ♀	None
4	5	Early unknown	250	60 ♂	None
13	11½	"	300	60 ♀	None
15	1½	"	180	52 ♂	None
23	1¾	11	115	38 ♀	Confluent bronchopneumonia
33	2½	11	390	32 ♂	None
38	3	11	325	35 ♀	None
18	2	12	100	50 ♀	Thrombosis of internal carotid and malacia cerebri
26	3	12	190	52 ♂	Symmetrical gangrene of toes. Mural thrombi of aorta
28	14½	12	160	44 ♂	Confluent bronchopneumonia; bilateral
30	2	12	270	52 ♂	Bronchopneumonia
6	10	13	340	40 ♂	Bronchopneumonia
17	3	13	225	32 ♀	None
24	3	13	160	46 ♀	None
34	2	13	110	77 ♀	Confluent bronchopneumonia
16	3	14	160	29 ♂	Thrombosis of superior mesenteric artery
29	1¼	14	225	29 ♂	Confluent bronchopneumonia
32	2	14	325	27 ♂	None
2	10	15	175	60 ♂	Bronchopneumonia
10	1½	15	165	50 ♀	None
21	2½	15	150	37 ♀	Confluent bronchopneumonia
36	2	15	60	60 ♀	Extensive decubitus
25	1½	16	175	37 ♀	Confluent bronchopneumonia
12	1½	18	100	47 ♀	Empyema. Phlegmon of leg. Impetigo of back
27	8½	18	190	55 ♂	Confluent bronchopneumonia
11	4	19	160	49 ♂	None
20	1¾	20	175	43 ♀	None
31	10	24	125	52 ♀	Confluent bronchopneumonia

marrow was present in our earliest autopsies (seventh day) in the form of small red areas. The volume of erythroblastic tissue seems to increase with the progress of the disease and cases of fourteen days and longer usually presented completely red marrows.

(h) *The respiratory tract:* Some degree of bronchitis and bronchopneumonia were present in thirty-seven of our autopsied cases. In eight cases extensive (confluent) bronchopneumonia was the apparent immediate cause of death. There were no distinctive appearances of the bronchopneumonic lesions, or of the lungs as a whole, which could serve to distinguish the process from secondary bronchopneumonia in general.

(i) *The alimentary tract:* We found no characteristic pathology of the alimentary tract and the esophagus, stomach, and intestines, with but few exceptions, showed no gross lesions. Shallow ulcerations of the stomach and duodenum were found in two cases. In appearance and situation these ulcerations were like those encountered frequently in other infections than typhus. Intense hyperaemia and punctate hemorrhage of various portions of the intestines and stomach was occasionally found as in deaths from other causes.

(j) *Salivary glands:* The sub-maxillary glands in one case were the seat of an acute suppurative exudate into the ducts and contained small abscesses.

(k) *The pancreas:* No acute changes were found in the gross.

(l) *The liver:* Focal necroses visible to the unaided eye were found in three instances, giving appearances in no way distinguishable from those occurring in other infections. In each of these cases there were other factors than typhus, respectively, phlegmon of the leg, suppuration of the sub-maxillary gland, and confluent bronchopneumonia. In the other autopsies no gross changes were found.

(m) *Urinary tract:* The kidneys in three cases only showed acute gross changes, once pyelitis and twice the changes usually called cloudy swelling. In these two latter instances the kidneys were above normal weight, and were pale and bulged

from the capsules when incised. There were no important complicating infections in these cases, and the parenchymatous changes were probably secondary to the lesions of typhus. The ureters showed no lesions. The bladder in a few cases only showed marked injection or minute hemorrhages into the mucosa or sub-mucosa. No lesion of importance was found in any case.

(n) *Genitalia:* The skin of the external genitalia shares in the lesions attending the eruption of typhus. No gross lesions were seen in the internal genitalia of either sex in any case; this is in marked contrast to the lesions of the male genitalia in Rocky Mountain spotted fever.

(o) *The skeletal muscles:* In no case in our series were gross lesions of the muscles found. The dry appearance of the muscles described by Aschoff (1915) is not substantiated by our experience. Waxy degeneration (Zenker's degeneration) was not seen by us in gross as described by Ceelen (1919). Minute necroses of muscle found microscopically are due to blood vessel lesions.

(p) *The serous membranes:* The pericardium, pleura, and peritoneum showed no changes attributable to typhus. We did not find the greasy condition of the peritoneum described by Aschoff (1915). In a few instances we found a marked dusky injection of the tendon sheaths in the ankles.

(q) *Ductless glands:* No gross lesions of the thyroid gland and pituitary body were found. The adrenal gland showed occasionally non-distinctive lesions such as are found in other infectious diseases — congestion and minute hemorrhages.

(r) *The central nervous system:* The gross changes observed by us were not striking; except in a few instances, where very marked injection of the blood vessels of the meninges and substance of the brain was noted. There were three cases where the degree of injection gave a pinkish coloration to the cortex of the cerebrum, and to the basal ganglia. In addition to these, three brains were slightly edematous. In four other brains the degree of vascular injection was pronounced. The degree of injection and edema of the brain has not corre-

sponded with the histological findings. Several brains showing the most extensive lesions histologically gave no gross evidence whatever of cortical lesions. We have concluded that the degree of injection and the presence or absence of edema of the brain found at autopsy is influenced by other factors, circulatory and toxic, and that the specific lesions of typhus do not necessarily markedly alter the appearance of the brain. Careful analysis of the autopsies fails to correlate any type of case or complication with the brains that were of striking appearance at autopsy.

One gross evidence of lesions due to typhus in the brain is the presence in section of the brain, particularly of the basal ganglia, pons and mid-brain of what are apparently prominently engorged blood vessels, which cannot be made to disappear upon pressure. These have proved to be small vessels surrounded by zones of hemorrhages into the perivascular "spaces."

3. PATHOLOGICAL HISTOLOGY

The first observation of a distinctive lesion in typhus was made by Fraenkel (1914, 1915) in skin removed from living patients. He described acute lesions of the blood vessels with thrombosis and perivascular accumulations chiefly composed of cells derived from the adventitia and peri-adventitial elements, but containing lymphocytes and polymorphonuclear leucocytes. These lesions he held to be specific for typhus. Fraenkel's findings and his conclusions in regard to the specificity of the skin lesions were confirmed by Aschoff (1915), Poindecker (1916), Ceelen (1916[1,2]), Bauer (1916), Kyrle and Morawetz (1916), von Chiari (1917, blood vessels of the conjunctiva), Jaffe (1918[1,2,3,4]), Herzog (1918), Kurt Nicol (1919), and a number of others. The same lesions were found in Mexican typhus in 1920 by Wolbach and Todd. Jaffe has perhaps written most extensively about the lesions in the skin.

Other lesions distinctive for typhus were found in the central nervous system by von Prowazek (1915), in man and animals

by Fraenkel (1915²), Aschoff (1915), Benda (1915), Ceelen (1916), Grzywo-Dabrowski (1918), and others. According to Ceelen the lesions in the brain were seen and described by Popoff in 1875 as circumscribed areas of cellular infiltration quite similar, when viewed under low magnification, to miliary tubercles. The most comprehensive descriptions of the central nervous system lesions are by Spielmeyer in 1919. Otto and Dietrich in 1918, and Doerr and Kirchner in 1919, found in the brains of guinea-pigs infected with typhus lesions similar to those previously found in man and monkeys.

A number of workers have found lesions in the heart, kidneys, and muscles of a similar nature to those described in the skin and central nervous system. While there has been difference of opinion in regard to the sequence of changes to be observed, and to the dependence of the perivascular reaction, both in cerebral and mesenchymal tissues, upon vascular lesions, the various authors have agreed in regard to the important lesions to be found histologically in typhus.

The admirable and complete review by Ceelen in the Ergebnisse of Lubarsch and Ostertag, 1919, makes it unnecessary to review the subject as a whole. Important papers will be referred to in connection with our descriptions.

(a) *The skin:* Our descriptions are based upon the study of skin from all parts of the body excised from thirty-six post-mortem subjects and from skin excised from the arm or abdomen, during life, from twenty-eight patients. From the autopsies we have material from the seventh to the twenty-fourth day of the disease. The skin excisions include material from the first day of the rash (third day) to the fifteenth day of the disease.

All descriptions are based upon sections stained with Giemsa's stain after Zenker's fixation. Acetic acid was used in the fixative in order to prevent the staining of mitochondria.

The presence of lesions is constant in all of our material. The combination of vascular lesions and perivascular accumulations (knötchen), forming a pathognomic picture, is present on and after the fifth day.

The only disease of which we know that gives a histological picture in the skin similar to typhus is Rocky Mountain spotted fever. The differences between the two are quantitative rather than qualitative. In Rocky Mountain spotted fever the destruction of the blood vessel walls is more extensive and the perivascular accumulations are less pronounced and do not form discrete tubercle-like nodules as in typhus. The vessel lesions, with the "nodules" described by Fraenkel and others, constitute the type picture in skin of typhus. (Plate XI, fig. 34 and Plate XIV, fig. 39.)

The diagnosis of typhus by microscopic examination of skin sections may be made, in our opinion, without finding the "nodules" of Fraenkel. The finding of lesions of the intima with thromboses in blood vessels in a disease with an acute exanthem other than Rocky Mountain spotted fever is sufficient. In very early cases and very late cases the blood vessel lesions may be overlooked; but we believe their presence to be constant. The "nodules," as Fahr (1915), Fraenkel, and Jaffe have insisted, cannot be found in every section. Serial sections are required. Of the twenty-eight skin excisions we failed to find characteristic perivascular "nodules" in four; and in these four cases only a few slides were made from the specimen. With the thirty-six autopsies from which skin was studied, the serial section method was not used, but many blocks of skin were taken from all parts of the body, — arm, shoulder, chest, abdomen, thigh, and ankle. Definite circumscribed infiltrations of the "nodule" type were found only in thirteen cases, but in most of the remaining cases the diagnosis of typhus could be made by one familiar with the histology of the disease.

The earliest reaction we have found (in skin excised the first day of the rash) consists of swelling of the endothelium of capillaries and small arteries and veins. The capillaries about coil glands and of the papillae are most extensively affected; but the vessels of the sub-papillary plexus also show reaction at this early stage. The swollen cells occlude capillaries completely and mitotic figures of endothelial cells

within capillaries and vessels of pre-capillary size are frequent. We believe that cells in mitosis outside of blood vessels are of endothelial origin as such cells can be seen fixed in the act of migration. At this early period polymorphonuclear leucocytes are fairly abundant in the connective tissue adjacent to vessels with lesions. Some of the swollen endothelial cells *in situ* (Plate XXVI, figs. 61 to 63 and Plate XXIX, figs. 70 to 73) possess a distinctive lightly stained appearance and when carefully examined by high power in well stained preparations, they are found to contain great numbers of minute paired lightly stained (blue) coccoid bodies (Plates XXVII and XXXII, figs. 67 and 80), identical in appearance to the rickettsia bodies in tightly packed cells of the louse's stomach (p. 137).

In skin taken in the fifth day of the disease, mural and occluding thrombi of fibrin are found in arteries and veins in the lower layers of the corium. Circumscribed groups of cells about vessels, the "nodules" (p. 159), are now present. (Plate XIV, fig. 39.) We believe that these nodules are invariably secondary to vascular lesions, or, that their location is always determined by lesions of blood vessels. We have many times seen vessels of pre-capillary size packed with swollen endothelial cells and in such instances the wall of the vessels is difficult to make out. Groups of occluded capillaries are to be found in the "nodules" of the sub-papillary region and in those replacing coil glands. The reaction about the coil gland plexuses results in the disappearance of the glands. The ensuing phagocytosis of degenerated epithelial cells by mononuclears complicates the picture but furnishes evidence that the majority of the mononuclear cells composing the early typhus nodule are macrophages, otherwise called mononuclear phagocytes and endothelial leucocytes. These cells in the typhus "nodule" are characterized by a lightly staining cytoplasm and give to the "nodule" a characteristic appearance. Paired granules which we believe to be rickettsia are frequently demonstrable within these cells in large numbers. Other cells found this early in the typhus "nodules" are

polymorphonuclear leucocytes and mast cells; the latter occur in increasing abundance as the disease advances. (Plates XIV and XV, figs. 39 and 40.)

Mural thrombi in arterioles and venules with very little perivascular reaction are found in the fifth day. The earliest indication of thrombus formation consists of masses of blood platelets massed upon and apparently within swollen endothelial cells *in situ*. Small fibrin thrombi apparently form upon such platelet collections while the underlying endothelial cells remain viable. We interpret the process as one affecting the cytoplasm of the endothelial cell in such a way, perhaps by a change in viscosity, as to attract the platelets. Whatever the interpretation, the fact is that microscopic mural thrombi are frequent and become at this early stage completely surrounded by endothelium. (Plate XIII, fig. 37.) The endothelial cells at the base of the thrombus frequently contain masses of rickettsia (Plates XXVII and XXVIII, figs. 83, 85, and 86).

Changes subsequent to the fifth day consist in a further development of the nodules and involvement of larger blood vessels, arteries, and veins in the lowest layer of the corium and in the subcutaneous fat. (Plate XII, fig. 36.) Occluding thrombi of large vessels are excessively rare; mural thrombi exist in vessels without severe damage to the media; indeed the muscular coat of blood vessels shows only the presence of migrating cells, and necrosis of individual muscle cells apparently secondary to the accumulation of migrating cells, mononuclear and polynuclear. Here again we have a contrast to the vascular lesions of Rocky Mountain spotted fever, where severe damage to the media occurs.

In addition to the "nodules," a diffuse perivascular infiltration is invariable in skin from typhus. The cells composing this diffuse infiltration are mononuclear phagocytes (endothelial leucocytes), lymphoid and plasma cells, mast cells, and rarely polymorphonuclear leucocytes. Mast cells are strikingly abundant. Polymorphonuclear leucocytes persist in fair numbers in the typhus "nodules" until reparative changes have begun. Their presence we believe is wholly in-

dependent of secondary infection and their number is determined by the abundance of degenerated cells, endothelial cells and connective tissue cells of the intima which become affected by the rapid accumulation of the reacting cells.

Even in fairly advanced cases, ninth to eleventh day, acute lesions of the intima may still be found in capillaries and small arteries and veins without marked perivascular reaction. (Plate XIII, figs. 37 and 38.)   Capillaries, venules, and arterioles with greatly swollen endothelium may be traced through many serial sections and cells containing rickettsia are more frequent than in the earliest days of the rash. The inference from an intensive study of the skin of all stages of the disease is that following the initial lesions due to the localization of rickettsia in the vascular endothelium, the subsequent spread of the organism excites a less marked cellular response, and may produce only a moderate reaction of the endothelium *in situ*.

Hemorrhages into the corium about capillaries and vessels of pre-capillary size are found on and after the eighth day. (Plate XII, fig. 35.) The mechanism of their occurrence seems to be a solution of continuity of the endothelium, due to degeneration and necrosis after the formation of thrombi in larger vessels has occurred, indicating that a mechanical factor — stasis — in veins is a necessary element. The occurrence of thrombi in the deeper vessels only at a later stage than the involvement of the superficial vessels suggests a centripetal extension of the infection. This we have actually shown to occur in particular cases by the study of the necroses over bony prominences where the condition is one of infarction involving skin and subcutaneous fat, due to the extension of thrombi facilitated by a moderate degree of stasis. Repair of the thrombi in the blood vessels occurs by organization and may be found as early as the fifteenth day. Repair of the perivascular nodules is difficult to follow. We have noted proliferation of the fibroblasts (connective tissue cells) at the periphery of the nodules on the eighteenth day and partial disappearance of the infiltrative cells with permeation

of the lesion by fibroblasts on the twentieth and twenty-fourth days. Lymphoid, plasma cells, and mast cells increase in number with the age of the nodule and phagocytosis of cells becomes more common. It is evident that repair takes place by fibrosis, by avascular granulation tissue.

(b) *The heart:* (Plates XVI and XVII, figs. 41 and 43.) The portions of the myocardium selected for microscopic examination from each case were the interventricular septum near the apex and the left ventricle wall at the base of and including the anterior papillary muscles.

Lesions are present in the slides of all of our cases, and with few exceptions of a degree and character making possible the recognition of processes due to typhus.

Slight degrees of edema evidenced by changes in the connective tissue septa and vacuolization of the muscle fibers is present in several cases.

The distinctive lesion is focal as described by Ceelen and Nicol and presents as a discrete area, smaller than the "nodules" in skin and brain and most often present in the inner half of the venticle wall. These lesions are most often in the substance of the myocardium and in our series rarely in the connective tissue septa. They consist of collections of cells in which large amoeboid and phagocytic mononuclears (endothelial cells) predominate; lymphoid and plasma cells are numerous and mast cells and eosinophiles are fairly common. Polymorphonuclear leucocytes are present in small numbers and in large numbers when there is necrosis of muscle fibers which is frequently the case in the area involved by this focal lesion. The necrosis usually involves only a portion of one or several muscle fibers. It is often impossible to recognize the obliterated blood vessels in these focal lesions, on the other hand capillaries filled with endothelial cells and frequently with fibrin thrombi are found in early lesions.

A more diffuse infiltration of the myocardium is invariably present in the form of endothelial cells, lymphoid cells, and plasma cells which lie packed between capillaries and (apparently) normal muscle fibers.

Focal infiltrations are found occasionally in the endocardium and in one case (Autopsy No. 18), in which there was thrombosis of the internal carotid artery, microscopic mural thrombi were attached to the endocardium in recesses between *columnae carnae*. (Plate XXV, fig. 59.)

In striking contrast to the skin, large blood vessels in the heart and pericardium very rarely contain lesions. A rare arteriole and venule contain mural thrombi in the whole of our series. The vascular lesions are almost wholly restricted to capillaries and vessels of pre-capillary size lying in the substance of the myocardium, and the vessels in the connective tissue septa have with rare exceptions escaped lesions. Small hemorrhages surrounding capillaries packed with endothelial cells and polymorphonuclear leucocytes is a rare finding.

The most extensive lesions were invariably present in the papillary muscle and adjacent ventricle wall. We have not succeeded in demonstrating rickettsia satisfactorily in the heart lesions. No groups like those found in the skin lesions were found. The endothelial cells concerned in the heart lesions contain so many inclusions that the occasional presence of one or several pairs of rickettsia-like bodies cannot be accepted with assurance.

(c) *The lungs:* Slides for microscopic examination were taken from portions of the lung most nearly normal as well as from portions with lesions. No lesions peculiar to typhus were found. In one instance only was there thrombosis of blood vessels. The bronchitis and bronchopneumonia in the stages seen in our post-mortem material cannot be distinguished from those attending other infectious diseases. The portions of the lungs free from pneumonic processes have shown no distinctive reaction to typhus.

(d) *The spleen:* There is no distinctive pathology. Only three spleens contain microscopic mural thrombi in the splenic veins (sinuses). One spleen from a case in which there was thrombosis of the superior mesenteric artery contained a thrombosed artery in a follicle. One spleen with a gross in-

farction due to thrombosis of an artery showed no evidence of bacterial infection.

The general changes in the spleen may be divided into three types. Early, late, and secondary to extensive confluent bronchopneumonia. The last may be disposed of with the statement that an excess of polymorphonuclear leucocytes is to be found in the blood vessels and reticular tissue of these spleens. The spleens from early cases show marked engorgement with blood and considerable phagocytosis of red blood cells by large mononuclear phagocytes. The degree of phagocytosis, however, is much less than that in typhoid and Rocky Mountain spotted fever. The follicles may or may not exhibit activity as evidenced by size and mitoses. The reticular tissue of the pulp is usually depleted of lymphoid cells, though plasma cells are abundant.

In late cases the marked engorgement is absent and there is very little phagocytosis. Brown granular pigment, presumably hemosiderin, may be abundant. The follicles are usually inactive. Many plasma cells are present at the periphery of follicles and in the pulp.

In spleens from both early and late cases the follicles may contain (eleven cases) a coarse hyaline fibrin-like reticulum in which polymorphonuclear leucocytes and mononuclear phagocytes occur. The presence of this material in the follicles bears no relation to the size of the spleen, presence of an important complication or stage of the disease. We regard it as a result of toxic action and its appearance is identical with the deposit in the follicles of spleens in many types of infection.

Acute lesions of the arteries and veins or the thromboses of typhus were not found in any of the thirty-seven spleens.

We did not find any evidence of myeloid transformation in the spleen as indicated by Nicol (1919, p. 138) who demonstrated that many cells in typhus spleens give the oxydase reaction. He also states that myelocytes can be seen in sections stained by Giemsa, — an observation which we fail to confirm. We did not employ the oxydase reaction in our study.

We have not succeeded in demonstrating rickettsia in the spleen to our own satisfaction. They may be present in cells lining the splenic veins (sinuses) but other granules render their recognition uncertain.

(e) *The liver:* In no case were lesions of large blood vessels found in the liver. An increase of cells in some portal spaces is common. These cells may fill and distend the space. They consist largely of lymphoid and plasma cells, though mononuclear phagocytic cells are numerous. This reaction seems to be a diffuse infiltration about small vessels rather than the "typical nodules" described by Fraenkel and Ceelen in livers. We have been unable to find thrombi in vessels in the portal spaces. The central veins (hepatic veins) show no reaction.

The most striking histological finding in the liver, described by Aschoff, Ceelen, Nicol, and others, and which we find to be practically constant, is a diffuse reaction on the part of the endothelial lining of the sinusoids — the Kupfer cells. These cells are in large number, swollen, occasionally in mitosis and very often with inclusions of red blood cells, leucocytes, pigment, and detritus derived from nuclei. Rarely, blood platelets can be recognized in these swollen cells. Mononuclear phagocytic cells, presumably detached Kupfer cells, are present in varying abundance in the sinusoids of the livers from all early cases. Patient search will usually also reveal a sinusoid filled with a fibrin thrombus and a rare degenerated or completely necrotic liver cell. Mitoses in liver cells as well as in the Kupfer cells are not rare.

The type of inclusion in the Kupfer cell described by Kuczynski (1918) as rickettsia from frozen sections does not appear to be present in our cases. Da Rocha-Lima's (1917, 1919, p. 297) failures to stain rickettsia in tissues is also contrary to Kuczynski's demonstration of rickettsia in the liver.

Bodies consistent with the rickettsia observed by us in skin vessel lesions and in lice sections are present in but small numbers in Kupfer cells and therefore are of little confirmatory value.

Focal necroses of a few to several liver cells were found in four livers and central necrosis in five. The latter condition, in three instances, was associated with important complications, phlegmon and impetigo in one case, confluent bilateral bronchopneumonia in two cases.

(f) *The gastro-intestinal tract:* Sections were made from the stomach, duodenum, jejunum, ileum, and colon as a matter of routine. In no case, exclusive of the case with superior mesenteric artery thrombosis, were lesions of large blood vessels found. The ulcers found in the stomach in two cases have no distinctive histology. In two other cases shallow ulcers of the stomach appear to have taken origin in areas of hemorrhage limited to the mucosa. In one of the above cases with ulcers a vein with a mural thrombus in the submucosa was found. Vascular lesions were conspicuously absent in all parts of the alimentary tract. Thrombi in blood vessels of the submucosa of the stomach were found in three cases; in the small intestine in another three cases and in the colon in one case. These findings agree with the rarity of gross lesions at autopsy.

(g) *The pancreas:* No lesions of the pancreas referable to typhus were found in the thirty-three examined. There were two pancreases that showed invasion of the interlobular connective tissue with polymorphonuclear leucocytes; a not uncommon finding in our experience with acute infections of various sorts. Two showed slight chronic pancreatitis. Lesions of the blood vessels of the pancreas are absent.

(h) *The kidneys:* Characteristic focal lesions in the kidneys and small hemorrhages have been described as of almost constant occurrence by Aschoff, Ceelen, Jaffe, and others. These lesions in our experience do not exhibit the proliferative reaction seen in the "nodules" of the skin and central nervous system. They do resemble the lesions in the myocardium in that only small blood vessels are affected and that the cellular response is infiltrative rather than proliferative. The lesions consist of small collections of cells incorporating one or several tubules which show varying stages of degeneration up to complete disintegration and necrosis. In many instances it is

easy to demonstrate thrombosed capillaries or blood vessels of pre-capillary size in these lesions. The cells consist of mono-nuclear phagocytic cells (endothelial leucocytes), lymphoid cells, plasma cells, mast cells, and eosinophiles in small num-bers and polymorphonuclear leucocytes. The lesions are most common in the pyramids but occur also in the cortex, where they are often in contact with glomeruli. Capillaries adjacent to or involved in the lesions are often packed with endothelial cells and lymphoid and plasma cells. Hemorrhage into the interstitial tissues and into tubules of the pyramids are con-stantly present in kidneys with these acute lesions. Twenty-six cases of our series showed these lesions. In the cases that did not show them are represented post mortems from the seventh to the eighteenth day of typhus.

We found no lesions of large blood vessels in the kidney. In one case there were small mural thrombi in small arteries in the sub-mucosa of the pelvis of the kidney and in this case both the large and small forms of rickettsia were found in groups in the endothelium of the affected vessels.

Lesions of the glomeruli in the form of an intracapillary pro-liferation of the endothelium were present in nine cases, in one case they were sufficiently marked to be accompanied by degeneration of the convoluted tube and to warrant calling the condition a glomerular-nephritis. This case died on the twentieth day of the disease and was one with unusually severe cerebral lesions. The glomerular reaction cannot be regarded as characteristic of typhus. We have observed the same re-action in other infectious diseases, recently in a series of post mortems upon influenza cases.

The adrenals: No lesions distinctive of typhus were found in the thirty-seven cases. In no instance were vascular lesions found. Twenty-six adrenals showed lesions of the cortex com-mon to infectious diseases in general. These lesions show as small areas of disappearance of the cortical cells, glomerular and fasicular zones, with accumulations of small numbers of endothelial leucocytes, lymphoid, and plasma cells. (Plate XVII, fig. 44.)

(i) *The bladder:* In one case out of thirty-seven a mural thrombus was found in a vein. No other lesions attributable to typhus were found.

(j) *The thyroid:* Thirty-four thyroids were studied. In one case only there was a thrombus in a fair sized artery. No other lesions attributable to typhus were found.

(k) *The blood vessels:* The aorta was studied microscopically from thirty-four cases. In three, blood vessels in the adventitia showed lesions with slight perivascular infiltration. We failed to find the lesions described by Ceelen as reminiscent of syphilitic aortitis; Nicol also failed to do so in twenty aortas from typhus cases.

Small areas beneath the endothelium filled with large vacuolated cells (fat-containing phagocytes) and occasional polymorphonuclear leucocytes were found in thirteen aortas. These lesions represent acute foci of degeneration of the intima and are common in many infectious diseases.

Another type of minute lesion was found in ten cases and probably is specific for typhus. This lesion consists of a layer of mononuclear cells, large mononuclear phagocytic cells, and plasma cells lying immediately beneath the endothelium, usually only one row deep and not associated with atheromatous processes. The large mononuclear phagocytic cells (endothelial leucocytes) of this lesion occasionally contain inclusions indistinguishable from rickettsia.

A special study of the left coronary artery was made in four cases of nine, ten, eleven, and eighteen days' duration of the disease. No lesions were found.

The femoral artery was examined in six cases; in two there were minute mural thrombi and in one of these rickettsia were found.

The femoral vein was examined in four cases; in one only was there a minute mural thrombus.

The superior mesenteric artery was examined in eleven cases; in ten there were no lesions, in the case (p. 154) in which there was thrombosis in gross no clue to the origin of the thrombus was found and the parts of the artery selected for sectioning

showed lesions common to thrombi remote from their points of origin.

The renal artery and vein were examined especially in five cases with negative results.

In one autopsy in which a gross thrombus was noted in the internal saphenous vein the sections show mural thrombi in all respects identical with those found in the skin vessels in typhus.

The lesions noted above of large blood vessels all seem to take origin in the endothelium and consist essentially of clusters of swollen endothelial cells on or in the intima usually accompanied by clusters of platelets and fibrin. These small mural thrombi are regarded by us as specific typhus lesions, and similar lesions presumably were the points of origin of the gross thrombi noted at post mortem.

(l) *The lymph nodes:* The inguinal and mesenteric lymph nodes were studied microscopically from every case. No constant changes attributable to typhus were found. The inguinal nodes in most instances contain an excess of mononuclear phagocytic cells (endothelial leucocytes) in their sinuses; in four cases this was very pronounced. Inguinal lymph nodes from two cases (seventh and eleventh day) contain thrombosed arterioles like those found in the skin.

The mesenteric lymph nodes with few exceptions were negative. In one from a fifteen day case without remarkable features an arteriole in the capsule is thrombosed. In a few instances, accumulations of phagocytic cells in the sinuses comparable to those in the inguinal nodes were found.

(m) *The bone marrow:* The marrow from the mid portion of the femur was studied histologically in thirty-two cases. In every case there is some degree of blood forming activity. The degree of activity apparently bears no fixed relation to the duration of the disease but in a general way there is more complete myeloid change in the marrow from late cases. The histological picture in all of these cases is practically that of normal active bone marrow. The marrow in general cannot be distinguished from that of any secondary anaemia and all of

the histological elements of normal marrow are present in proportions within the normal range. In a few cases with extensive bronchopneumonia there are increased numbers of polymorphonuclear leucocytes and granular myelocytes present. Megakaryocytes are present as in normal marrow.

In the marrow of one case a small artery contains a thrombus. In a few marrows there are in addition to the usual blood forming activity an increased phagocytosis of erythrocytes and in capillaries and venules accumulations of phagocytic mononuclear cells (endothelial leucocytes).

An observation perhaps of interest is that in the four marrows showing the earliest degrees of activity the presence of small groups of marrow cells, pre-myelocytes and myelocytes, is usually attended by the presence of delicate fibrin strands and the fat cells are separated apparently by a liquid which has yielded a finely granular precipitate. This we regard as probably a regular concomitant of the early development of activity.

(n) *The skeletal muscles:* (Plate XVI, fig. 42.) Pieces of muscle were sectioned for histological study from thirty-six cases. The muscles selected for examination were the biceps, rectus abdominalis, and quadriceps extensor group.

Lesions of the blood vessels were found in all cases but one. The vascular lesions are like those of the skin. In one case with early thrombi in an artery rickettsia were found in abundance in the endothelium. Extensive perivascular accumulations like those in the skin are the rule with vessels containing lesions. A diffuse infiltration, like that in the myocardium, of lymphoid, plasma cells, and eosinophiles between muscle fibers is common but not constant. Degenerated muscle fibers in or adjacent to the perivascular lesions are common; the appearances of these individual fibers is that of Zenker's degeneration, and in late cases we have regenerative changes in the sarcolemma.

In one case there was fairly extensive waxy degeneration (cf. p. 157) (Zenker's degeneration) of the rectus muscle. This case was one with extensive bronchopneumonia, and the

blood vessels of the muscle show no unusual degree of injury. The discrete degenerated fibers we are inclined to regard as the result of the vascular injuries.

The involvement of large blood vessels in the skeletal muscle is second only to that in the skin and testes; although the lesions in muscle are not as striking as those in the central nervous system.

(o) *The female genitalia:* The breast was studied histologically in six cases without finding lesions.

The uterus was studied histologically in six cases without finding lesions.

The ovaries in the sixteen cases studied were negative. The Fallopian tubes in five cases studied were negative.

(p) *The male genitalia:* The skin of the scrotum in the fourteen cases studied shows lesions of the blood vessels corresponding in number and degree to those occurring in the skin of other parts of the body.

The prostate glands from sixteen cases studied show no lesions in the blood vessels or glands referable to typhus.

(q) *The testes and adnexa:* (Plate XIII, fig. 38.) Vascular lesions (thrombosis) are present in the testis or epididymis or both in all of the sixteen male cases studied histologically. Perivascular accumulations like those of the skin of a proliferative nature are present in most of these cases. In five cases rickettsia can be satisfactorily demonstrated in the blood vessel lesions of the testis or epididymis.

In seven cases there was a considerable degree of aspermatogenesis, evidenced by absence of spermatozoa and diminution in the number or complete absence of mitoses. In two cases the aspermatogenesis is complete in some portions of the slides and is attended by slight hyaline thickening of the basement membrane of the tubules. These two cases were not attended by severe vascular lesions. One was complicated by extensive bronchopneumonia. We regard this change in the testes to be independent of local lesions and to a general effect of the disease. Similar aspermatogenesis has been observed by one of us in epidemic influenza and by Mills in epidemic pneu-

monias (Jour. Exp. Med., Baltimore, 1919, Vol. XXX, No. 5, p. 505) caused by the streptococcus and pneumococcus.

(r) *The central nervous system:* The lesions of the brain and spinal cord in typhus have been extensively studied by the authors (Ceelen, 1916, Spielmeyer, 1919) cited above. The occurrence throughout the central nervous system of nodules or of tubercle-like lesions, in some way associated with blood vessels, is regarded by all as constant and characteristic. (Plates XX, XXI, and XXV, figs. 49, 50, 51, 52, and 60.) Other lesions not characteristic but constant are perivascular infiltrations with lymphocytes, plasma cells, and macrophages and infiltration of the leptomeninges with similar cells. Nicol, Ceelen, and Spielmeyer have recently (1919) given detailed descriptions of the histological findings in the brain and all of the essential points are well established by their work. Spielmeyer has entered into the most detailed account and in some minor points has arrived at opinions at variance with ours.

The typhus nodule in the central nervous system is widely distributed, more abundantly in gray matter than in the white. It appears characteristically as a sharply circumscribed lesion the size of a small miliary tubercle; it is without necrosis and composed almost wholly of cells unanimously regarded as neuroglia cells. (Plates XVIII to XXI inclusive.)

We examined the brains from all of our typhus autopsies, thirty-seven in number. The stages of the disease are shown in Table XVI.

Owing to the large volume of work undertaken by us, a complete topographical study of the brain was not undertaken. Material, however, was saved as a routine from the following locations: cerebral cortex including frontal, parietal, temporal, and occipital lobes; basal ganglia including the optic thalamus, caudate nucleus, lenticular nucleus, and usually the internal capsule; cerebellum including cortex from the lateral lobes and dentate nucleus; mid-brain usually through the anterior corpora quadrigemina; the pons; and the medulla at the levels of the decussation, the olivary bodies and through the middle of the fourth ventricle. The Gasserian ganglia and pituitary

body were also examined as a matter of routine in twenty-seven cases. Zenker's fluid was the fixative employed. The staining methods were Giemsa's as used by us for other tissues and Mallory's phosphotungstic acid hematoxylin for neuroglia. Giemsa's stain possesses unusual merits we believe as a routine stain for the central nervous system. Ganglion cells and neuroglia cells, with their processes, are picked out with a sharpness equal to Nissl staining, and the cytoplasmic bodies, Nissl granules, etc., are shown with equal clarity. All of the distinguishing features and differential staining of wandering cells, lymphoid cells, plasma cells, eosinophiles, mast cells, and macrophages are present. Non-medullated nerve fibers appear as delicate bluish stained filaments. The sheaths of medullated nerves are stained pinkish. Finally, by the employment of thin paraffin sections, it is possible to trace out the ramifications of the small capillaries, and to study the endothelium of blood vessels of all sizes.

Lesions were found in every brain in our series. The impressiveness of the skin rash noted in life corresponds fairly well with the degree of cerebral involvement noted in the histological study as Ceelen has observed. However the degree of the rash which persisted post mortem was not in a general way related to the extent of involvement of the brain. There is also a definite correspondence between the severity of mental and motor disturbances shown by the patients and the number and distribution of the cerebral lesions found in the histological study. These observations are shown in Table XVI. Motor disturbances, such as twitching, trismus, *cerea flexibilitas*, we associate with extensive involvement of the cerebral cortex. Marked mental symptoms cannot be associated with any distribution of the lesions, but are associated with a general extensive cerebral involvement. The few cases of marked cardiac disturbances seem definitely to have been associated with extensive lesions of the medulla and probably are in part the effect of capillary hemorrhages.

The tabulation of the distribution of the proliferative lesions (Table XVI) is roughly accurate. We have not been able to

TABLE XVI.   PROLIFERATIVE LESIONS IN THE BRAINS OF TYPHUS PATIENTS

Case No.	Duration Days	Cortex Cerebri	Caudate N.	Lenticular N.	Optic Thalamus	Mid-brain	Medulla	Pons	Cerebellum	Ammon's Horn	Capillary Hemorrhages	Nervous Symptoms noted in Life	Rash as noted in Life
8	7	+++	+++	+++	+++		+++	+++	++		Medulla	Marked trismus, subsultus tendinum, delirium, coma, meningismus	++
19	7	++	+	+	+	++	+++	++	−	+ −		Pons. Cortex cerebri	
35	8	+ ++	−	− −	− −		+ + + +	++ +++	− + −	+ −			+++
3	9	++ +	+ −	+	− −	++		+++					
7	9				++								
9	9	rare ++	−	−			++	++	−	−		Medulla	Delirium; coma
1	10	++					++	++	−	−			++
14	10	++	+	+		+	++	++	+	+ −			Vaso-motor collapse, fifteen hours before death. No muscular signs. Coma only six hours before death
22	10	++ +	+ −	+ − −	− −	− + +	++ +++		− − −	−	+ −		Anxiety, slight stupor. No motor disturbances. No delirium	+
37	10	+	−	−	−	+	+	+++	−	−	−		
4	Early unknown					+		++	−				Stupor only	+++
13	Early unknown	+	−	−	−	+	+	+	−	+	−		
15	Early unknown	+++	+	+	+	++	++	+	++	++	+	Medulla	
23	11	+	+	−	+	++	++	++	+	++		Caudate nucleus	Delirium, coma, retention of urine. No motor disturbances	+++
33	11	+	−	−	−	+	++	+++	−	−	−		Noisy, delirious, convulsions preceded death	+++
38	11	++ +++	+	+	+	++	++	++	+	++	+ +	− Thrombosis of internal carotid	Noisy, delirious; sudden death	++
18	12								− +				
26	12	+++	++	++	++	++	++ +++ +++	++	++	++			

No.	Crosses by region								Region	Clinical notes	
28	12	+++	++	++	++	++	+++	++	—	Delirium; coma, meningismus. No motor disturbances	+++
30	12	+++	+	—	—	+	+++	+++	Cerebellum.	Slight delirium; trismus on last day. Sudden coma and collapse	++
6	13	+++	+++	+++	+++	+++	+++	+++	White matter	Trismus; coma. Pharyngeal paralysis?	+++
17	13	+			—	—	++	—	Optic thalamus. Pons. Medulla	Restless. No delirium. Sudden terminal collapse	+++
24	13	+	+	+	+	+	++	++	Ammon's Horn	Excitable; irritable. No delirium. Sudden terminal collapse	++
34	13	++++	++++	++	++	+++++	+++++	+++	Medulla	Marked cardiac irregularity. Slight delirium. Stupor. Retention of urine	+++
16/29	14/14	+++/++	++	++	++	++/+	++/++	+	—	Slight stupor. Muscular resistance to passive motion	+++
32/2	14/15	++/++	++	+/—	+/—	++/++	++/++	+	—	Stupor; coma. No motor disturbances	++
10/21/36	15/15/15	++/+++++/+++	++/—	++/+	+++/++/+	++++/++/++++	++++/+++/+++	+++	—	Slight delirium only	+
25	16	+	+	+	+	—	+	+++	—	Moderate delirium. Retention of urine. No motor disturbances	+++
									—	Moderate delirium. Stupor. Coma. No motor disturbances	+++
12	18	+	+	++	++	+++	+	++	Cerebrum. Sub nigra (mid-brain). Dentate N. (cerebellum)	Marked delirium. No motor disturbances	+++
27	18	+++	+	+		+	+	++	—	Delirium; stupor; meningismus; trismus	+++
11	19	+++	+++	+++		+++	+++	++	Sub-ependymal medulla		
20	20	+++	++	+++	+++	+++	++	+++	—	Long continued coma. Trismus. Irregular heart action	+++
31	24	+	+	+	++	+	+		—	Drowsy; partial coma. Incontinence of urine. No motor disturbances	+++

NOTE: The crosses indicate presence and roughly the relative number of the lesions. The results are based upon the examination of several sections from one block of tissue from each region. No entry means that no slide was prepared. The cases without clinical notes were not studied in our wards.

plot the relative frequency of the lesions in different cortical areas, and the only positive conclusion upon this subject that we have made is that the parietal lobe leads in abundance of lesions and the occipital lobe shows fewest.

The medulla and pons probably show the most numerous lesions. We agree with Ceelen and Nicol that sections through the medulla including the olivary bodies offer the best chance of discovering these lesions; they are particularly numerous in the olivary nuclei. We have counted fifty-two lesions in a single section through the medulla at this level. The pons comes next in our estimation; the lesions are most abundant in the gray matter (nuclei pontis) and central gray matter, as elsewhere in the brain. The mid-brain and basal ganglia in our estimation come third as seats of predilection for these lesions, then follow the cerebral cortex, Ammon's horn, and cerebellum.

The proliferative lesions are found in the white matter, but much less frequently than in the gray. In the cerebrum it is difficult to find them in the white matter while in the medulla and pons they occur with frequency in the fiber tracts. In one case only was a proliferative lesion found in the internal capsule (case 20). In the cerebral cortex the lesions occur in all layers; but they are most numerous in the middle group of ganglion cell layers, second to fifth. In the basal ganglia, the thalamus, caudate nucleus, and lenticular nucleus are equally susceptible. In the cerebellum, the molecular layer and the dentate nucleus are most often the site of lesions; next in order come the Purkinje cell layer, the granular layer, and white matter. In the medulla, the olivary nuclei are most heavily affected. Lesions in the central gray matter of the fourth ventricle just below the ependyma are common. At the level of the decussation of the pyramidal tracts lesions are always numerous.

The occurrence of capillary hemorrhages usually restricted to the perivascular space is shown in Table XVI, and are probably of importance in the production of cardiac and respiratory symptoms.

The choroid plexus from each of the thirty-seven brains was

examined microscopically, and no lesions of any sort were found. Ceelen, however, reports the finding of typical typhus "nodules" in the choroid plexus.

The histology of these proliferative lesions has received much study by Ceelen (1919), Nicol (1919), and Spielmeyer (1919). All are agreed that the lesion is primarily a reaction on the part of the neuroglia. Both Ceelen and Nicol concluded that the proliferative lesions take origin in lesions, or at the site of lesions, in capillaries and pre-capillaries, a conclusion which we unreservedly corroborate. Spielmeyer believes that the lesions arise independently of processes localized in blood vessels. He describes four types of lesions all proliferative in character. The common or typical lesion he calls the compact lesion. This type is the small tubercle-like lesion, which usually extends through seven to eight 15-micron sections in series. They measure 0.1 to 1.5 microns in diameter, though much smaller ones are found. His rosette forms are small lesions extending through two or three sections only and are formed by rod-shaped and sausage-shaped neuroglia cells distributed radially about capillaries. This type of lesion is most common in the superficial layers of the cerebral and cerebellar cortex.

A lesion practically limited to the molecular layer of the cerebellum, though occurring in the superficial layer of the cerebral cortex, is described by Spielmeyer as "strauchartiges" or a "Gliastrauchwerk" (bush-like or glia bush work). This lesion consists of a diffuse neuroglia increase in small areas, about 0.1 mm. in diameter, and was found in distinctive form in but one case, though present to some degree in four other cases in his series of thirteen brains. His fourth type of lesion is that called "Gliastern" (glia star) and consists of a single or double layer of glia cells surrounding a pre-capillary. The cells may be radially arranged and thus the lesion may resemble a small rosette lesion. The glia stars are small, extend through one or two 15-micron sections and are commonest in the pons and spinal cord. The glia stars and the glia bush work (Gliastrauchwerk) lesions are not restricted to typhus, but are found also in typhoid fever.

In our series we found with regularity lesions of the rosette and star types in the locations specified by Spielmeyer. The "Strauchwerk" lesion was not present in typical form as illustrated by Spielmeyer in any of our human cases. In the molecular layer of three cerebellums we found small areas of diffuse neuroglia increase in each instance associated with marked capillary dilatation in the same area. In the cerebellums of guinea-pigs we have frequently found lesions simulating closely the illustrations of Spielmeyer's "Strauchwerk" lesions and here also we have demonstrated that the network of neuroglia cells giving rise to this picture are apposed to very fine capillaries. In guinea-pigs mitotic figures are not uncommon in neuroglia cells lying in contact with the capillary walls. We feel imposed to emphasize the delicacy of the capillaries in the ganglion cell layers of the cerebral cortex and in the molecular layer of the cerebellum and the possibility of failing to identify capillaries even in thin sections when not injected with red blood cells. The capillaries to which we refer have apparently lumina permitting the passage of single red blood corpuscles only. In the thick sections employed by all of the German investigators details involving capillaries might escape notice.

The histology of the type lesion of typhus in the central nervous system, the compact lesion of Spielmeyer, the nodular lesion of others, was quite accurately described by von Prowazek, who recognized that neuroglia cells were an important constituent of the nodule. Polymorphonuclear leucocytes, rare endothelial cells, plasma cells, and rod cells were all included by him. Benda, Ceelen, Nicol, and Spielmeyer all emphasize the rôle of the neuroglia cell in the formation of the nodule. The presence of polymorphonuclear leucocytes, plasma cells, and rod cells is described by each of the above authors. Spielmeyer states specifically that the amoeboid neuroglia plays no part in the formation of the lesion.

We have devoted much time to the study of these very interesting lesions in the human brain and have controlled our observations by a study of the brains of thirty guinea-pigs, showing these lesions. The animal material proved particu-

larly valuable in deciding the relationship of vascular lesions to the proliferative lesions. Our observations are in the main confirmatory of the authors named above. We are, however, not willing to accept without reservation the dictum that cells of mesenchymal origin do not invade the brain substance in the formation of lesions. The identification of wandering cells in the central nervous system, in spite of a voluminous literature, still presents great difficulties as reliable criteria for the recognition of the various types of cells have not been formulated. We believe that the wandering mononuclear phagocyte (macrophage, endothelial leucocyte, etc.) does enter nervous tissue, and is an important constituent of the typhus "nodule" in the brain.

Our study of human brains from early typhus deaths and of guinea-pig brains secured from all stages of experimentally transmitted typhus shows that the first evidence of typhus is found in capillaries and pre-capillaries in changes to be observed in the endothelium. Swollen endothelial cells and endothelial cells exhibiting degenerative changes in nucleus and cytoplasm, often accompanied by small platelet and fibrin thrombi, are found in the brains of guinea-pigs killed on the first or second day of fever. (Plates XXII, XXVIII, and XXXI, figs. 53, 59, and 77.) Similar vascular lesions in company with fully developed lesions are frequent in human brains from patients dying before the tenth day of typhus, as various stages of the evolution of the brain lesions are constantly found together both in human and animal material. (Plates XXI, XXII, and XXIII, figs. 51, 54, and 56.)

Capillaries, when obliterated by the proliferation of endothelial cells, often appear bloodless and therefore of doubtful identity; the presence of one or several blood corpuscles taken up by the detached endothelium has frequently served to settle doubt as to the presence of a damaged blood vessel. Escape of red blood corpuscles is frequent and small perivascular hemorrhages are constantly seen in both guinea-pigs (Plate XXII, fig. 53) and human brains (Plate XXIV, fig. 57) about vessels of pre-capillary size.

The next stage in the development of the lesion is the appearance of mononuclear cells around the affected capillary or pre-capillary. When the vessel wall remains in evidence, as it frequently does, as a delicate pink staining hyaline structure, these mononuclear cells seem to have an extra-vascular origin. We have frequently seen cells which are universally regarded as perivascular neuroglia cells in mitosis immediately adjacent to thrombosed capillaries. On the other hand, the occurrence of phagocytic cells early in the formation of the lesion reveals the presence of macrophages (endothelial leucocytes) and we believe that these cells of mesenchymal origin play an important rôle in the early lesion.

The reaction of neuroglia seems almost simultaneous with that of the endothelium, and the small nodules consist of both of these elements. (Plate XXII, fig. 54.) Polymorphonuclear leucocytes are invariably present in small numbers. In the adjacent brain tissue we find at this early stage the presence of elongated cells directed radially towards the lesions, cells which by virtue of their shape and granular cytoplasm are identified as types of neuroglia cells, the "rod cells." Satellite cells in apposition to ganglion cells immediately adjacent to the lesions show swollen nuclei; and forms indicative of migration towards the lesion are common. There is also an increase in number of satellite cells in the zone adjacent to the lesion and ganglion cells involved in the periphery of the "nodule" undergo neuronophagia. The lesion increases in size by the accession of mononuclear cells with abundant cytoplasm. Many of these cells have shapes and processes indicative of neuroglial origin; and with the phosphotungstic acid hemotoxylin stain the cytoplasm appears homogeneous and to contain, near the nucleus, prominent centrosome-like bodies in pairs and clusters. With Giemsa's stain the cytoplasm of these cells appears in many instances to be filled with minute deep blue staining granules. Whether rickettsia bodies are included cannot be proved, as the cytoplasmic granulation cannot be differentiated from that found in neuroglia cells in other types of reactions.

It is very often impossible to outline individual cells in these compact lesions, and we are convinced that there is a syncytial formation. (Plates XXI, XXII, and XXV, figs. 52, 54, and 60.) Fibrils cannot be demonstrated in relationship to these cells except in late lesions at the periphery where their presence leaves open the question of their origin; i.e., whether from the surrounding neuroglia or from the cells of the nodule.

The number of polymorphonuclear leucocytes may be great, exceeding that of other cells. Migration of polymorphonuclear leucocytes can be seen from capillaries remote from the lesions. Occasionally the number of polymorphonuclear leucocytes suggests in appearance a pyogenic infection; but this reaction we believe to be called forth by the necrosis resulting from complete thrombosis of several adjacent capillaries.

Lymphoid and plasma cells are absent in the vast majority of lesions. Rarely a cell corresponding to a lymphoid cell is encountered in a lesion. We have not seen typical plasma cells in lesions, although Giemsa's stain is well adapted for their identification, as can be demonstrated in the perivascular zones of infiltration in the majority of the brains studied. Plasma cells at the periphery of the lesions are encountered, but in relationship to capillaries.

Rod cells arranged radially in the tissue surrounding these lesions are quite common and we believe enter into the formation of the lesion. The lesions do not increase in size beyond the dimensions given by Spielmeyer, i.e., 0.1 to 0.12 millimeters. The various stages of their formation may be seen in the same brain and until the febrile period in typhus has passed.

We have not been able to study these lesions in human brains later than twenty-four days after the onset of typhus; and in guinea-pigs have not yet studied the brains of late recovered cases. Nicol has found the lesions to persist as late as the eighth week, and has found recognizable traces, i.e., cicatrices three to four months after the onset of typhus. He describes the healing of most of the lesions as taking place, without

leaving cicatrices, by degeneration and migration of the cells. But a few cicatrices are left in the form of spindle shaped cells (neuroglia ?). Our own observations lead us to believe that the lesions following the disappearance of the cells are replaced by small amounts of fibrillary neuroglia.

Other smaller loose-textured lesions are common, both in human and animal tissues in the superficial layer of the cerebral cortex (Plate XXIII, fig. 55) and in the molecular layer of the cerebellum. (Plate XXIV, fig. 58.) Some of these lesions we identify as the rosette lesions mentioned by Spielmeyer, others take the form of irregularly arranged, many processed, and apparently anastamosing glia cells occupying areas 0.05 to 0.1 millimeters in diameter and arising we believed by the proliferation of pericapillary neuroglia cells.

These various lesions of the central nervous system represent a definitely proliferative reaction preceded by injury to and probably always by a proliferation of the endothelium of capillaries and pre-capillaries. The lesions are composed chiefly of two elements, cells derived from the vascular endothelium and from the neuroglia. The lesions are invasive in character in that they lie in the substance of nervous tissue. Vascular lesions determine solely the sites of these proliferative lesions. The evidence at hand points to the conclusion that the endothelial and neuroglial proliferation is in direct response to the presence of the parasite of typhus, carried we believe into the nerve tissue by the migration of endothelial cells. The actual demonstration of rickettsia in the endothelial cells of blood vessels *in situ* in the brain has been accomplished by us in human and animal tissues. The presence of fine granules in the neuroglia cells of the lesions makes the recognition of rickettsia in this location uncertain, though in the guinea-pig there are masses of paired granules within cells and lying apparently between cells which we believe are rickettsia. Clusters of bodies like that illustrated in Plates XXVIII and XXX, figures 69 and 75, we accept without reservation as rickettsia.

Hemorrhages in the central nervous system in typhus are usually limited to the perivascular spaces. Occasionally, how-

ever, there is infiltration of the nerve tissue with blood with resultant small foci of destruction. Such lesions always contain many macrophages filled with red blood corpuscles. (Plate XXIV, fig. 57.)

In all brains, some degree of infiltration of the pia arachnoid and some degree of perivascular infiltration (Plate XIX, fig. 48) is present. We have never found proliferative lesions in the meninges. The perivascular and meningeal infiltration is most marked in cases of considerable duration and probably reaches a maximum at the end of the second week. As a rule, the more numerous the proliferative lesions, the more extensive are the perivascular and meningeal infiltrations, which apparently represent a general response to the pathology of typhus rather than a local response due to localization of the virus of the disease.

The perivascular reaction is widely distributed, and we have failed in our attempts to correlate it with degree of meningeal infiltration or variation in the distribution of the proliferative lesions. The perivascular infiltration consists chiefly of lymphoid and plasma cells, though macrophages (endothelial leucocytes) and mast cells are not uncommon. A fibroblastic proliferation has not been seen in our material. Polymorphonuclear leucocytes, and rarely an eosinophile, are found. An increase in the perivascular neuroglia is present in marked degrees of infiltration.

The most marked degree of perivascular infiltration is to be found in the basal ganglia, pons, and medulla.

The infiltration of the pia arachnoid is not diffuse. It occurs in areas in all locations, most constantly and abundantly over the cerebellum, pons, and medulla, and is independent of proximity of either the proliferative lesions or perivascular infiltration in the substance of the brain. The cells found are chiefly macrophages (endothelial leucocytes), lymphoid, and plasma cells. Small hemorrhages are also occasionally found in the arachnoid and their ante-mortem occurrence is shown by the phagocytosis of the blood corpuscles by macrophages.

Ganglion cells in the brain apparently undergo complete destruction only when incorporated in areas involved by proliferative lesions. Neuronophagia has been seen by us in all parts of the cerebrum and cerebellum. In addition, in nuclei in the medulla and mid-brain and in the Purkinje cells of the cerebellum we have seen striking changes in the ganglion cells — chromolysis and axonal reaction — probably exhaustion effects. These observations have been made on tissues removed within two hours post mortem and must represent changes which occurred in life. However, we have not undertaken a detailed study of the ganglion cell changes in typhus, as they are not peculiar to this disease. The possibility of the persistence of the proliferative reaction in the central nervous system after subsidence of the temperature or apparent duration of the disease is suggested both by the late deaths of guinea-pigs with paralytic symptoms and by the continuation of severe nervous symptoms in man leading to death long after the fall in temperature.

Experiments recently begun with guinea-pigs indicates that the virus of typhus does survive in the brain after the temperature has become normal. We have had numerous histological indications that this is the case; we have found early cerebral lesions in apparently recovered animals. The following protocol shows that the virus is present in the brain at least on the third day of normal temperature.

Record of guinea-pig 387, inoculated May 25, 1921 from guinea-pig 376 (see Chart 1, patient 116 strain).

Temperatures:

	°F.			°F.
May 25	103.0	June 6		104.2
" 26	102.7	" 7		104.1
" 27	102.9	" 8		104.7
" 28	104.6	" 9		104.2
" 29	No record	" 10		104.6
" 30	102.9	" 11		104.2
" 31	No record	" 12		No record
June 1	103.4	" 13		104.2
" 2	103.8	" 14		102.5
" 3	102.7	" 15		102.7
" 4	103.3	" 16		102.4
" 5	No record			

The guinea-pig was killed June 16th. The autopsy was negative except for a slightly enlarged spleen. Histological examination of the brain showed numerous lesions of typhus in the cerebrum and cerebellum.

One of the three guinea-pigs inoculated with an emulsion of the brain of guinea-pig 387 reacted in a manner typical of typhus, and the others showed less marked febrile reactions at the same period.

Record of guinea-pig III D inoculated with brain emulsion of guinea-pig 387:

	°F.			°F.
June 16	102.7	June 28		103.2
" 17	103.0	" 29		103.8
" 18	102.7	" 30		103.2
" 19	No record	July 1		103.7
" 20	102.3	" 2		102.8
" 21	102.8	" 3		No record
" 22	102.6	" 4		No record
" 23	103.3	" 5		104.5
" 24	103.6	" 6		105.0
" 25	104.3	" 7		No record
" 26	No record	" 8		105.2
" 27	103.0	" 9		105.6

The guinea-pig was killed on July 9th for diagnosis. The autopsy showed an enlarged spleen covered with a thin fibrin-like layer. The peritoneum as a whole was normal in appearance. The other organs were normal. Histological examination of the brain showed many typical typhus lesions.

(t) *The pituitary body:* The pituitary body from twenty-one cases was studied histologically. No lesions of blood vessels or parenchyma were found in the anterior or glandular lobe. Vascular and proliferative lesions of the posterior lobe were found in eleven cases. Nowhere else in the body is there an equally striking illustration of the selective localization of the typhus virus, and an explanation why blood vessels in the posterior lobe are affected while those of the anterior lobe escape involves the general consideration of the pathology of nerve tissues.

The proliferative lesions in the pituitary resemble those in the brain; but the participation of the neuroglia is beyond

actual demonstration. The number of phagocytic cells having the appearances of ordinary macrophages indicates that many of the cells are of endothelial origin. The relationship of the proliferative lesions to capillaries is easily shown here as elsewhere. Capillaries with endothelial lesions and thrombosis are common. Perivascular accumulations of lymphoid cells, plasma cells, and phagocytic mononuclear cells (macrophages, endothelial leucocytes) occur in the substance of the posterior lobe and in the dural investment.

(u) *The Gasserian ganglion:* One Gasserian ganglion was sectioned from each of twenty-six cases. Lesions due to typhus were found in fifteen, these lesions include capillary lesions with and without adjacent proliferative lesions, perivascular infiltrations and reactions of the capsular cells following ganglion cell degeneration.

The proliferative lesions are in the nerve trunks as well as in the ganglion cell areas, and are composed of cells indistinguishable from many cells composing the "compact" (p. 180) type of lesion in the brain. These cells are probably of endothelial origin and they frequently contain inclusions of other cells. Perivascular accumulations of lymphoid cells, plasma cells, and phagocytic mononuclear cells (macrophages, endothelial leucocytes) with rare polymorphonuclear leucocytes and mast cells are found in the nerve trunks and the arachnoidal investment of the ganglion.

Degenerative changes in ganglion cells are common and are attended by swelling and proliferation of the capsular cells. Complete disappearance of ganglion cells leaving a residuum of Nissl granule dust in spaces nearly filled by proliferated capsular cells is frequent in ganglia with extensive lesions.

The proliferation of capsular cells and degeneration of ganglion cells is not unlike that of rabies, though less striking. A diffuse infiltration with lymphoid cells, plasma cells, and macrophages of considerable areas of the ganglion is common and such areas usually include one or several degenerated ganglion cells with capsular proliferation.

(v) *The demonstration of rickettsia in human tissue:* Early in the study of the vascular lesions paired, ovoid, somewhat lanceolate bodies were found in considerable numbers in endothelial cells *in situ.* (Plates XXVIII and XXXII, figs. 68, 79, and 81.) These bodies are slightly smaller in size than those in Rocky Mountain spotted fever, but in other respects they are similar. They are found only in the endothelium and never, as in Rocky Mountain spotted fever, in the smooth muscle of the media. They are usually surrounded by a narrow clear zone or halo and the pairs measure slightly over 1 micron in length and 0.2 to 0.3 microns in width. (Plates XXIX and XXXII, figs. 70, 73, and 82). They correspond to the larger and more deeply stained pairs found in the louse. When present in endothelial cells in numbers these larger forms of paired rickettsia are arranged in rows following the long diameter of the cell. (Plates XXVII, XXVIII, and XXXII, figs. 66, 68, 79, and 81.)

A more extended study showed that cells containing large numbers of these deeply staining forms were rare, but that on the other hand cells with one to several pairs were common. (Plate XXIX, fig. 73.)

Smaller paired forms, massed within cells as described in Mexican typhus (Wolbach and Todd, 1920), were found in nearly every case carefully studied (Plate XXVI, figs. 61, 62, and 63). This globular massing of the organisms is the most characteristic appearance of rickettsia in human lesions. (Plates XXVII, XXVIII, XXXI, and XXXIII, figs. 65, 69, 78, and 87.)

The organisms stain with difficulty and it is sometimes necessary to vary the technic of staining in regard to the reaction of the water used for dilution of Giemsa's stain and in regard to the degree of differentiation in order to get satisfactory preparations. Frequently swollen endothelial cells, with circumscribed lightly staining granular areas, were found upon restaining to contain masses of rickettsia occupying the areas found appearing as lightly staining and granular. In our

notes we referred to this appearance, for a considerable period of our research, as gray stippling of endothelial cells. We now conclude that the appearance is due to masses of the most minute form of rickettsia because of our ability to definitely stain them and because of their frequent association in the same cell with the larger more deeply stained form (Plate XXIX, figs. 71, 72, and 73). Cells massed with diffusely distributed rickettsia occur in the endothelium adjacent or surrounding thrombi as well as in vessels showing no lesions other than swollen endothelium. In contracted arteries and veins these swollen cells project into the lumen and are often filled with uncountable numbers of paired granule-like rickettsia.

Occasionally, we have been able to see short straight or curved rod-like forms in association with the other forms (Plate XXXII, fig. 83), but for the present we distinguish with certainty two forms only, a larger, relatively deeply staining, paired, lanceolated form and a smaller, lightly stained, paired, coccoid form in globular masses or diffusely distributed in endothelial cells.

In searching for rickettsia in lesions it is necessary to practice much patience as the vagaries of staining and possibly of distribution make their demonstration a matter of fortune unless a system of searching is followed. (Plate XXXIV, figs. 88 and 89.) Our method has been to locate lesions in vessels and then to follow each vessel in both directions from the lesion through the short series of sections which it has been our practice to make from each block of skin. The best results in staining are probably obtained by partially differentiating the sections in alcohol and completing the differentiation after mounting in oil of cedarwood by exposure to sunlight.

The finding of rickettsia in the mononuclear cells of the perivascular "nodules" and the evidence that these cells multiply in the nodules as shown by mitoses lead us to the conclusion that this reaction is almost wholly a proliferative one and caused by the presence of the parasite of typhus in these cells. It also seems evident that the rickettsia are carried

from the blood vessels by migratory endothelial cells. The analogous lesion in the central nervous system, particularly in guinea-pigs, hardly permits of any other explanation.

(w) *Recapitulation of the vascular lesions:* The earliest effect is upon the endothelial cells, producing swelling and proliferation and a degree of injury resulting in thrombosis. Perivascular collections of cells result from the further proliferation of cells which we believe to be induced by the presence of rickettsia within cells of endothelial origin. Other cellular reactions occur as in other disease processes; there is accumulation of polymorphonuclear leucocytes, lymphoid and plasma cells, and mast cells. Eosinophilic leucocytes are present in the perivascular lesions in a few of our cases.

The presence of bodies indistinguishable from rickettsia has been demonstrated in twenty-one of the twenty-eight pieces of skin excised ante-mortem. Our failures with this material is directly attributable to faulty technic in embedding and staining.

Of the autopsy material, rickettsia have been found in the skin of twenty-five cases which represents every case where the post-mortem examination was made before the thirteenth day of the disease and while the body was in a fresh condition. In two cases rickettsia were found as late as the fifteenth day. Other organs in which we have demonstrated rickettsia to our own satisfaction are kidneys two cases, femoral vein one case, testes and adnexa five cases, and the brain seven cases. We have given a relatively short time to the search for rickettsia in the central nervous system as compared with that devoted to the skin in the search for organisms. Owing to the small size of the involved vessels in the brain and the presence of many granule bearing cells of neuroglia and endothelial origin which make difficult the recognition of endothelial cells *in situ* in capillaries, we have preferred to depend upon the skin lesions for our numerical evidence in regard to the constancy of rickettsia in typhus lesions.

The possibility of mistaking granules of various sorts for rickettsia impels us to state our criteria for identification.

Situation is of first importance — the localization in endothelial cells, which do not contain granules of any sort except in stages preliminary to division. We find that the granules stainable by Giemsa's stain in endothelial cells about to undergo or in mitosis are of varying size and irregular shapes, but it has been our practice to disregard such cells in the search for rickettsia. In questionable instances, moving to the section next in series settles the question of nuclear activity. The granules in mast cells stain differently and more deeply than rickettsia, a deep purple or red in contrast to the pale blue of the latter (Plate XXIX, figs. 73 and 74). They are larger than rickettsia but are often paired and may be removed from the nucleus as this cell is extraordinarily amoeboid. Mast cells rarely penetrate vessels. The invariable use of a series of sections mounted upon one slide always enabled us to trace out the delicate processes of amoeboid mast cells to their nuclei. Pigment cells are so distinctive that the possibility of mistaking their granules for rickettsia does not exist for the experienced histologist.[1]

Other inclusions phagocytized by endothelial cells are larger, of irregular shapes and staining reaction.

Our control material includes a large variety of pathological skin conditions, infarctions, burns, X-ray radiation, petechiae in septicaemias, measles, scarlet fever, chicken-pox, and Rocky

[1] A paper published by Stevenson and Balfour, since the completion of our manuscript, in the Journal of Pathology and Bacteriology, July, 1921, describes and illustrates granules in human tissues which the authors regard as consistent with rickettsia. They do not, however, express an opinion as to the "true nature" of the granules.

The distribution of the granules, noted by Stevenson and Balfour (ganglion cells of the brain, larger vessels of the lungs, phagocytic cells of the spleen, the parenchyma and Kupfer cells of the liver, pancreatic cells, kidney cells, and intestinal gland epithelium), does not coincide with the distribution of rickettsia as described by us in this report.

The fixative used in preserving the tissues of the five cases studied makes impossible an exact comparison of their findings with ours. We have briefly discussed on page 191 our criteria for the recognition of rickettsia in tissues. Since Stevenson and Balfour found rickettsia-like (?) bodies in the parenchyma cells of several organs where we did not find them, and since they failed to find them in the endothelium of blood vessels of the skin where in our experience rickettsia are most numerous, we are forced to regard their technic and criteria for identification of rickettsia in tissues as unreliable.

They failed to note that in a preliminary report (1920) we recorded the presence of *Rickettsia prowazeki* in the lesions of typhus in human and animal tissues.

Mountain spotted fever, so that we can state with assurance that the bodies described as rickettsia in typhus lesions are peculiar to typhus and are indistinguishable from rickettsia as seen in the infected louse.

In chicken-pox material recently provided by Dr. James Denton perivascular accumulations of cells about the papillary capillaries and vessels of the sub-papillary plexus bear a resemblance to the lesions of typhus. The vascular lesions seem to be limited to swelling and proliferation of the endothelium without thrombosis. The changes in the epidermis preceding and coincident with vesicle formations preclude confusion with typhus.

XII

PATHOLOGICAL HISTOLOGY IN GUINEA-PIGS

1. Description of Tissues

(a) *The central nervous system:* In regard to the proliferative lesions of the brain, the vascular lesions, perivascular and meningeal infiltrations are like those in human brains. Stage for stage the processes are identical, and involve the same cells as in the human. The proliferative lesions of the "compact" type are perhaps relatively less numerous than in man, while the perivascular grouping of radially directed neuroglia cells, "rosettes" of Spielmeyer and the diffuse proliferation of neuroglia in apposition to capillaries in the molecular layer of the cerebellum and in the superficial layer of the cerebral cortex ("Strauchwerk lesion of Spielmeyer") (page 179) are relatively more numerous. All these types of lesions are to be found in cerebrum, cerebellum, and medulla distributed much as in man.

These central nervous lesions in guinea-pigs are constant. They may be so numerous as to cause paralysis (Plate XVIII, figs. 45 and 46) and death or so few as to require sections from several parts of the brain for their detection. Otto and Dietrich (1918) were the first to describe them in the guinea-pig and to indicate that they could be recognized in frozen sections. We have found these lesions in the brains of thirty-six guinea-pigs in a consecutive series; and unhesitatingly take the position that their presence is an indispensable criterion for the proof of typhus in the guinea-pig. In guinea-pigs killed in the first forty-eight hours of temperature, only vascular lesions may be present, although we have had but one such instance. For the early diagnosis, i.e., before the third day of temperature, we recommend paraffin sections, for later periods frozen sections stained with methylene blue suffice for the recognition of the compact lesions.

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The series of thirty-six guinea-pigs include the brains of guinea-pigs inoculated directly from man, from monkeys, with the intestinal tracts of lice and from guinea-pigs. We have never seen lesions of the choroid plexus in guinea-pigs. The lesions in the guinea-pig are more easily found in the cerebral cortex than in the pons, medulla, or cerebellum.

In the guinea-pig (No. 219) referred to on page 145, which died of paralysis, proliferative lesions are very numerous; eighty-one are present in a single cross section of the cortex of the cerebrum. (Plate XVIII.) Small perivascular hemorrhages are also present in this case, a rare finding in the brains of typhus guinea-pigs.

The histo-pathology of the remaining organs is like that in man, though much less striking.

(b) *The skin:* In the skin of the scrotum and ears we have found vascular lesions and perivascular infiltrations like those in man; but the proliferative lesions leading to nodule formation is not so prominent a feature. Thrombi are rare in the skin. The endothelium forms large groups of cells, which remain attached to the vessel wall; and many of the cells in such masses are phagocytic. A concentric thickening of the intima due to proliferation of the endothelium to the extent of two or more layers of epithelium-like cells also occurs. The perivascular nodules often contain mitotic figures and contain very few polymorphonuclear leucocytes. They appear to be almost pure collections of macrophages, i.e., endothelial leucocytes.

(c) *The heart:* Proliferative lesions are rare; but occur adjacent to blood vessels in the myocardium. Occasional small perivascular infiltrations with lymphoid cells, plasma cells, macrophages, and granular leucocytes can usually be found in a single section through the ventricles.

(d) *The lungs:* Rarely mural thrombi of arteries and veins and occluding thrombi of capillaries have been found. These lesions are not accompanied by striking reactions in the adjacent tissues. The accumulations of phagocytic endothelial cells in the capillaries of the alveolar walls in restricted areas

is probably the result of typhus. Heaping up of the endothe-
lium of arteries and veins with perivascular accumulations of
the same type of cells (endothelial) as those in the skin lesions
are also frequently found. On the whole, vascular reactions are
more common in the lungs of guinea-pigs than in those of man.

(e) *The spleen:* The spleen is usually markedly engorged,
the splenic follicles are large with conspicuous germinal cen-
ters, containing numerous mitoses. There is always an increase
in large cells in the pulp, with much phagocytosis. We regard
these cells as endothelial in origin. In the majority of the
spleens megakaryocytes and hematopoietic cells are absent.
In a few cases, occasional erythroblastic groups and mega-
karyocytes are found. In the splenic veins or sinuses are many
phagocytic mononuclear cells (endothelial leucocytes, macro-
phages) enclosing red blood corpuscles and nuclear remains of
other cells. Polymorphonuclear eosinophiles are present in
increased numbers in the pulp, and neutrophilic polymor-
phonuclears are present in small numbers. The peritoneal
cells on the surface of the spleen are usually swollen. The
spleens on which a thin transparent fibrin-like layer was noted
fail to show evidences of acute infection. The layer upon the
surface consists of a delicate, compact, fibrinous material cov-
ered on both surfaces with cuboidal cells. These cells are evi-
dently derived from the peritoneal epithelium, as the cells
upon the surface of the spleen are identical in appearance and
always contain many cells in mitosis. Mitotic cells are also
found upon the surface of the fibrin, with rarely a phagocytic
cell, and very rarely a polymorphonuclear leucocyte. The
histology of the spleens with this surface layer is not different
from spleens free from such coating, and, in the absence of
any reaction in the capsule, it is difficult to regard this material
as exudate from the spleen caused by secondary infection.

Smear preparations have repeatedly shown no bacteria and
cultures on ordinary media were negative.

(f) *The liver:* The lesions in the liver are insignificant but
similar to those in man. There is an increase in size of the
endothelium lining sinusoids (Kupfer cells) and phagocytosis

by these cells of red blood corpuscles and white blood cells. Small clumps of proliferated endothelial cells occur as compact, discrete lesions. Occasionally in the portal spaces they are small, similar proliferative lesions composed of endothelial cells. Blood vessel lesions are not found. Large necroses, which are common in guinea-pig livers from many sources, were naturally found in a number of our typhus guinea-pigs and show no distinctive histology.

(g) *The gastro-intestinal tract:* The gastro-intestinal tract was studied microscopically from a few guinea-pigs. No lesions of typhus were found.

(h) *The pancreas:* No lesions of the parenchyma or blood vessels were found.

(i) *The kidneys:* The only lesions found in the kidneys are small collections of cells about capillaries in the medulla. These occur with great rarity in only a few kidneys. The cells are large and evidently of endothelial origin. Mitoses occur in such cell groups.

(j) *The adrenals:* No lesions were found.

(k) *The skeletal muscles:* No lesions were found.

(l) *The uterus and ovaries:* No lesions were found.

(m) *The testes:* The testes and adnexa are second only to the brain in number and typical character of the lesions. Vascular lesions, proliferative perivascular reactions and simple perivascular infiltrations are found in the testes, epididymis, tunica, cremasteric muscle, and polar fat. These lesions are comparable to those found in man and in the skin and subcutaneous tissues of the scrotum of the guinea-pig.

Arteries and veins of considerable size, as well as capillaries, are affected. Fibrin thromboses are rare, but occur. The commonest lesion is a mural thrombus composed of proliferated endothelial cells and such accumulations may almost occlude large vessels. A concentric thickening of the intima by endothelial proliferation also occurs. The perivascular proliferative reaction usually completely surrounds large and small vessels, including capillaries, and the cell most abundant is that which we have been calling endothelial leucocytes

(macrophage, large mononuclear phagocyte). Polymorpho-
nuclear leucocytes also are found in these lesions. The mural
thrombi and proliferative perivascular reactions are more
common in the tunica and epididymis than in the testis.
Lesions of the seminiferous tubules were not found.

Diffuse perivascular infiltrations are not a conspicuous
feature of typhus in the guinea-pig; the cells occurring in the
testes and adnexa are largely lymphoid cells and plasma cells
with occasional polymorphonuclear eosinophiles, neutrophiles,
and a rare mast cell. Neill's (1917) brief description of the
testicular lesions in guinea-pigs in our opinion overstates the
degree of involvement.

2. Rickettsia in Guinea-pig Tissues

Our successes in demonstrating rickettsia in guinea-pig
tissues have not been equal to those with human tissue,
partly owing to the fact that we have devoted but little time
to the attempt and partly because the character of the lesions
is milder in that they present fewer guides to the search.

Scattered paired coccoid bodies often can be found in swol-
len endothelium in blood vessels of the skin of the scrotum,
the testes, and the brain. (Plate XXX, fig. 76.) The more
minute form of paired bodies in large numbers in endothelial
cells of blood vessels in the same tissues are also usually to be
found if sufficient search is made in well-stained preparations.
The capillaries and vessels of pre-capillary size of the brain,
with early lesions, are the most favorable fields in the search
for rickettsia, and here only have we found the compact
groups of paired organisms, such as are found in human blood
vessels. (Compare Plate XXVIII, fig. 69, from a guinea-pig's
brain with Plates XXVII and XXXIII, figs. 65 and 87, from
human material.)

If we are correct in our interpretations of the various forms
of rickettsia in human tissues, to the effect that the minute
forms represent the early forms, the short duration of the
disease in guinea-pigs would explain the rarity of the clusters
of the larger forms of rickettsia.

XIII

SUMMARY AND CONCLUSIONS

WE have already stated in the introduction that our results are in general confirmatory of observations made by widely separated workers and that the work which was independently planned by us serves as a carefully conducted control to a number of researches made under less favorable circumstances in several countries.

Our experiments with lice were the first to be made with lice from sources absolutely unquestionable, both as to the occurrence of rickettsia-like micro-organisms and the presence of the virus of any disease. The fact that these lice when nurtured upon typhus patients acquired only *Rickettsia prowazeki*, in the way of demonstrable micro-organisms, with great regularity (cf. table, p. 44) is in itself very strong evidence for the etiological relationship of *Rickettsia prowazeki* to typhus.

The experiments described, with protocols, under the heading of "Experiments to Prove the Specificity of *Rickettsia prowazeki* for Typhus" (p. 51) admit of no other conclusion than that the virus of typhus is not separable in the louse from *Rickettsia prowazeki*. We could not devise means of determining why lice do not invariably become infective when nurtured under proper conditions upon typhus patients. It would appear from our results that an element of chance enters into the infection of the louse with *Rickettsia prowazeki* (the virus of typhus). Possibly the louse in order to become infected must pierce a capillary at the site of a lesion or localization of rickettsia in the endothelium. This idea implies that the mechanism of typhus transmission is an imperfect one in more than one respect, for it must be remembered that infection with *Rickettsia prowazeki* eventually destroys the louse

which is an unusual effect of a parasite upon its intermediate host.

We were also unable to experiment upon the manner of introduction by the louse of rickettsia into the human. We are certain that *Rickettsia prowazeki* escapes from the alimentary tract with the feces and therefore may be introduced by scratching or by the mouth parts of the louse after becoming soiled with feces. We have not seen rickettsia in the salivary glands or in the esophagus of a louse.

Incidentally, through the accident which befell Mr. Bacot, we obtained important evidence that the extracellular rickettsia, present in a considerable per cent of the lice in Warsaw, are *Rickettsia pediculi* and the cause of trench fever.

Our pathological studies, combined with the demonstration of rickettsia in the lesions, add to the clearness of our conception of typhus as a disease, as well as to the proof of the causal relationship of rickettsia.

The lesions of typhus are located in the blood vessels of the skin, central nervous system, skeletal muscles, and to a lesser degree in a few viscera, heart, kidneys, and testes. Hence, we may say that typhus is a disease of the smaller blood vessels; and we have demonstrated that the parasite of the disease localizes almost exclusively in the vascular endothelium. The reaction to the parasite (*Rickettsia prowazeki*) is shown primarily by degenerative changes, giving rise to thromboses in blood vessels, and by a proliferative reaction on the part of endothelium and neuroglia which give rise to the characteristic microscopic "nodules" of the disease in skin and central nervous system. Death from typhus in man is frequently the direct result of extensive involvement of the brain with the proliferative lesions, in guinea-pigs the rare fatality due to typhus infection is usually caused by the cerebral lesions. That the virus of typhus is probably present in the cells of the proliferative lesions is shown by the fact that we have transmitted the disease by the injection of an emul-

sion of the brain of a guinea-pig (p. 186) three days after the return to normal temperature, a period when the blood is believed to be no longer infective.

We have demonstrated rickettsia in human and guinea-pig tissues. In human tissues they have been found in blood vessels in the skin, brain, kidneys, muscles, and testes, in fact in all tissues the blood vessels of which become the sites of lesions. As far as we know, physiological knowledge indicates no differences between the blood vessels affected by typhus and those not, by which the selective localization of *Rickettsia prowazeki* can be explained.

The demonstration of *Rickettsia prowazeki* in lesions demands careful attention in matters of technic and tissues not materially changed by post-mortem processes, and above all rigid control. As there is no specific stain for *Rickettsia prowazeki* the following criteria must be satisfied: (1) the size, (2) morphology, (3) staining reactions must correspond with those of *Rickettsia prowazeki* in sections of lice, and (4) they must be present in vascular endothelial cells *in situ* in relation to the lesions of typhus.

We have avoided presenting as evidence observations made upon cells with secretory activities and nerve and neuroglia cells, and cannot accept such observations by others as bearing upon the question of the relationship of rickettsia to typhus.

We have not included in this report the fruitless attempts we have made to cultivate *Rickettsia prowazeki* from guinea-pigs. It does not seem possible to us that any one of the bacteria cultivated from typhus patients and experimental animals can be *Rickettsia prowazeki*. The staining reactions and morphology of *Rickettsia prowazeki* from lice furnish criteria yet to be met by any culture. Our inability to preserve the infectivity of typhus blood under a variety of conditions at incubator temperature for more than four days is strong additional evidence that none of the reported bacteria can be *Rickettsia prowazeki*.

We conclude that *Rickettsia prowazeki* is the cause of typhus. Our most important evidence is that contained in the experiments from which we have concluded that the virus of typhus and *Rickettsia prowazeki* are inseparable in infective lice. Next in importance is the fact of the presence of bodies indistinguishable from *Rickettsia prowazeki*, demonstrable with great regularity, in the lesions of typhus in man.

XIV

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XV

DESCRIPTION OF PLATES

THE drawings were made by Miss Etta R. Piotti to whom we express our thanks and appreciation.

All of the illustrations except Fig. 10 of Plate II were made from preparations, sections, or smears, stained with Giemsa's stain.

The drawings are accurately made to scale with the camera lucida. Unless otherwise stated, the photomicrographs are exactly the number of diameters specified.

PLATE I

FIGURE 1. Louse Box 60, Louse 9. *Rickettsia prowazeki.*

2,000 diameters. Smear. This preparation is made by the dissection of the gut of an experimentally infected louse. In the field are many rickettsias. They appear as granules and short rods disposed singly or in pairs. The irregularity of size and staining of the organisms forming the pairs is clearly shown. There are also in the field short chains composed of two or more paired organisms.

FIGURE 2. Louse 4 from Dr. Weigl. *Rickettsia rocha-lima.*

2,000 diameters. Smear. This preparation was made from a louse of the Commission's American stock which had been maintained on himself by Dr. Weigl. Such lice invariably became infected with the organism illustrated. To it Dr. Weigl has given the name *Rickettsia rocha-lima.* The organisms exist as granules or short rods which lie singly or in pairs. Their irregularlty in size and staining reaction is clearly seen. Among the rickettsias lie many organisms which are considerably above the average in size; such organisms, as well as the small ones, often unite to form short rods. Dr. Weigl recognizes pleomorphism as one of this organism's characteristics.

FIGURE 3. Mr. Bacot's stock. Louse 2. *Rickettsia pediculi.*

2,000 diameters. Smear. This preparation was made on the tenth day of Mr. Bacot's attack of trench fever (see page 130) with one of the stock lice maintained by him. The smear, made by the dissection and teasing out of the gut of the louse's viscera, shows many rickettsia bodies. The organisms occur as granules and short rods which are disposed singly or in pairs. One of the numbers of a pair is often smaller than the other and difference in staining between the chromatinic granules and ground substance composing the organisms is seen.

FIGURE 4. Polish control Louse 27. *Rickettsia pediculi?*

2,000 diameters. The rickettsia bodies illustrated were found (refer to discussion of Polish controls in text, page 130) in lice taken from apparently healthy inhabitants of Warsaw. The organisms occur as granules, singly or in pairs, and as short rods, singly or in pairs.

FIGURE 5. Bed-bug 2, Slide 1, Mr. Bacot's stock. *Rickettsia lectularius.*

2,000 diameters. This preparation is the extremity of a Malpighian tubule dissected from one of the stock of bed-bugs brought from England and maintained by Mr. Bacot. At one edge of the field lie cells of the extremity of the Malpighian tubule. They are closely packed with rickettsia bodies. Free in the field are many other organisms. The granules and short rods are disposed singly or in pairs.

FIGURE 6. *Dermacentroxenus rickettsi.*

2,000 diameters. From a smear preparation of an experimentally infected tick (Dermacentor venustus) twelve days after feeding on an infected guinea-pig. Tick LXVIII (Wolbach, 1919, p. 102).

PLATE II

FIGURES 7a, 7b, and 7c.

3,600 diameters. These three drawings illustrate rickettsia forms in a smear from the gut and gut contents of an experimental louse (Louse 10, Box 48). Some of the swollen threads, as 1c, shown are probably rickettsia forms. Nothing is stated concerning the nature of the remaining structures.

FIGURE 8. Louse Box 50, Louse 2. *Rickettsia prowazeki.*

2,400 diameters. Smear. The louse from which this smear of gut and gut contents was made came from a box of lice in which infection both by *Rickettsia prowazeki* and *Rickettsia pediculi* occurred. Thread-like chains of *Rickettsia prowazeki*, similar to those illustrated here, are not rare. In this drawing the unbroken outline of the bluish ground substance of the thread is indicated. Formed, apparently, within the substance of the thread are the purple granules indicating the poles of rickettsia bodies of varying morphology.

FIGURE 9. Louse Box 48, Louse 10. *Rickettsia prowazeki.*

2,400 diameters. Smear. There are very many rickettsia bodies of characteristic appearance in this smear of gut and gut contents; as is illustrated, they often clearly show purple staining poles and a mid-piece colored pale pinkish blue. In addition to these, threads of great length are present.

FIGURE 10. Louse Box 65, Louse 10. *Rickettsia prowazeki.*
1,200 diameters. Stained with cyanochin blue. The few rickettsia shown in this preparation are not stained. They appear as translucent bodies lying upon a colored background. When so demonstrated, the organisms give the impression of being somewhat larger than they seem to be in preparations stained by Giemsa's method. The diversity of form and size is well shown.

FIGURE 11. Louse Box 47, Louse 6. *Rickettsia prowazeki.*
1,200 diameters. Smear. Contents of louse's mid-gut; granular and bacillary forms are present.

FIGURE 12. Louse Box 43, Louse 4. *Rickettsia prowazeki.*
1,800 diameters. Smear. Contents of louse's mid-gut; bacillary forms, measuring 1μ to 2.8μ in length.

PLATE III

FIGURE 13. Louse Box 48, Louse 10. *Rickettsia prowazeki.*
1,200 diameters. Smear of a louse's mid-gut; granular and paired forms are seen as well as a tangled skein of rickettsia in thread-like chains from a squashed epithelial cell.

FIGURE 14. Louse Box 60, Louse 9. *Rickettsia prowazeki.*
1,800 diameters. Smear preparation from teased cells of the mid-gut. This illustrates the type form of rickettsia as usually described.

FIGURE 15. Polish Lice, Louse 19, Dr. Weigl. *Rickettsia prowazeki.*
The forms illustrated occurred in a smear preparation made with the gut and gut contents of one of a series of lice given by Dr. Weigl. Dr. Weigl stated that these lice had been infected with *Rickettsia prowazeki* by feeding upon typhus patients. In the group of rickettsia bodies illustrated the indefinite bluish stained ground substance and the purple polar or central granules are clearly seen.

FIGURE 16. Louse Box 57, Louse 42. *Rickettsia prowazeki.*
1,200 diameters. Section. The epithelial cells of the gut are greatly swollen and vacuolated. They are packed with enormous numbers of granular rickettsia. The organisms occur as fine granules singly or in pairs. Also present are larger, usually paired, rickettsia bodies which take a deeper stain. "Filaments" (see page 139), of varying size and stained a bright red, are seen in several places.

FIGURE 17. Louse Box 17, Louse W 231. *Rickettsia prowazeki.*
1,200 diameters. Section. Epithelial cell of louse's mid-gut filled with bacillary and paired forms. (See protocol, page 53.)

PLATE IV

FIGURE 18. Louse Box 58, Louse 14. *Rickettsia prowazeki.*

1,200 diameters. Smear preparation of the gut of a louse. This drawing illustrates rickettsia free and contained within epithelial cells. They occur as granules singly or in pairs. Among the paired forms are some of larger size.

FIGURE 19. Bed-bug caught in Warsaw. *Rickettsia lectularius.*

1,800 diameters. Smear showing a burst Malpighian tubule. Very numerous rickettsia, varying in morphology from single granules to short paired rods.

PLATE V

FIGURE 20. Mr. Bacot's stock, Louse 2. *Rickettsia pediculi.*

2,000 diameters. Section. Characteristic granules, disposed singly or in pairs, occurring free in the lumen and, especially, closely placed along the cuticular border of the epithelial cells of the gut. (Compare with Figure 32, plate IX, which is from a different field.)

FIGURE 21. Louse Box 58, Louse 14. *Rickettsia prowazeki.*

2,000 diameters. Smear. Epithelial gut cells from an experimental louse are shown. They are closely packed with finely granular rickettsia bodies. (Compare with Figure 18, plate IV.)

PLATE VI

FIGURE 22. Louse Box 48, Louse 10. *Rickettsia prowazeki.*

2,000 diameters. Smear. The epithelial cells of the mid-gut have been squashed in making this preparation; their contents are spread over the slide. Many rickettsia, as single or paired granules or as short single or paired rods, are scattered about the field. A tangled mass of chain-like rickettsia "threads" fills a large part of the field. (Compare with Figure 13, plate III.)

FIGURE 23. Louse Box 54, Louse 2. *Rickettsia prowazeki.*

2,000 diameters. Section. This epithelial gut cell is closely packed with rickettsia bodies. At one end of the cell the organisms are present in a granular form; at the other end of the cell they appear as rods which lie more or less regularly ordered in skeins. Among the finely granular organisms lie others, singly or in pairs, which are considerably larger and stain more deeply. (Compare with Figure 29, plate IX.)

PLATE VII

FIGURE 24. Louse Box 60, Louse 2. *Rickettsia prowazeki*.

Low power, photomicrograph. Section. This longitudinal section through an experimentally infected louse shows the mid-gut filled with débris from uncompleted digestion of blood. Most of the epithelial cells of the mid-gut are apparently normal. Some of them are swollen and distended. Such cells are infected with *Rickettsia prowazeki*. They are very characteristic and can be easily recognized by examination under the dissecting microscope.

FIGURE 25. Louse Box 64, Louse 3. *Rickettsia prowazeki*.

2,000 diameters. Low power, photomicrograph. Sagittal section through an experimentally infected louse. The mid-gut is filled with débris of partially digested blood. Some of the epithelial cells are apparently normal. Most of them, especially at the anterior end of the gut and the diverticulum (D), are heavily infected with *Rickettsia prowazeki* and are greatly distended and swollen. Such an appearance of the epithelial cells is characteristic of heavily infected lice. Such cells are recognizable under the dissecting microscope.

PLATE VIII

FIGURE 26. Louse Box 57, Louse 4. *Rickettsia prowazeki*.

1,200 diameter. Section of a louse's mid-gut. Enormously swollen epithelial cells are shown. The clear areas probably represent collections of cell secretion. The remainder of the cells are filled with rickettsia bodies single and paired.

FIGURE 27. Louse Box 54, Louse 2. *Rickettsia prowazeki*.

Low power. Section of the mid-gut of a louse. In only one small area are the epithelial cells of the gut unswollen. Everywhere else the epithelial cells are swollen, sometimes they are greatly swollen. All the cells are heavily infected with rickettsia. The cell marked "A" is illustrated at higher magnification in Plate IX, figure 29. The two cells marked "B" in Plate IX, figure 31.

PLATE IX

FIGURE 28. Louse Box 62, Louse 4. *Rickettsia prowazeki*.

1,200 diameters. Section. Epithelial cell of a louse's mid-gut. Contains rickettsia of varying forms. Cocci, diplococci, and rod forms, singly and in short chains, are present.

FIGURE 29. Louse Box 54, Louse 2. *Rickettsia prowazeki*.

1,200 diameters. Section. A swollen epithelial cell of a louse's mid-gut. This is an illustration, at a higher magnification, of the cell marked "A" in Plate VIII, figure 27. One end of the cell is filled with a granular mass of rickettsia among which occur scattered paired forms. At the other end of the cell the rickettsia, in chains, are characteristically disposed in a tress-like skein.

FIGURE 30. Louse Box 54, Louse 1. *Rickettsia prowazeki*.

1,800 diameters. Section. Swollen epithelial cell of the mid-gut containing bacillary forms of rickettsia. The organisms are usually arranged in skeins of thread-like chains. They also occur singly, in pairs, and in short chains. Lying free in the lumen of the gut is a short rickettsia thread.

FIGURE 31. Louse Box 54, Louse 2. *Rickettsia prowazeki*.

1,200 diameters. Section. Swollen cells of a louse's mid-gut. This is an illustration, at a higher magnification, of the cells marked "B" in Plate VIII, figure 27. Both cells are filled with minute rickettsia which occur singly or in pairs. Scattered throughout the cells are larger paired forms. The clear areas in these cells probably represent collections of cell secretion. Over such areas the forms of discrete rickettsia granules are clearly seen.

FIGURE 32. Louse Box 53, Louse 3. *Rickettsia pediculi*.

1,200 diameters. Section. The epithelial gut cells contain no organisms. Many riskettsia bodies are thickly placed over the surface of the cuticular border of the cells; this is well seen in one cell where, in sectioning, the cuticular border has been cut tangentially. The lumen of the gut is filled with partly digested blood. In the gut contents occur bacilliform crystals and spherical bodies which are colored with hemoglobin. Many rickettsia bodies occur everywhere in the lumen of the gut. They always occur as granules either singly or in pairs; they do not show the wide variation in form which is exhibited by *Rickettsia prowazeki*.

PLATE X

FIGURE 33. Louse Box 64, Louse 3. *Rickettsia prowazeki*.

1,200 diameters. Section. Junction of the esophagus and mid-gut of a louse. The epithelial cells of the esophagus are not infected. The epithelial cells of the mid-gut are greatly swollen and are heavily infected with rickettsia. Most of the intracellular rickettsia are granular. Near the base of the membrane of the gut, on either side of the esophageal orifice, are small groups of bacilliform rickettsia.

PLATE XI

FIGURE 34. Skin. Low power. From a case of Mexican typhus showing the proliferative lesions "typhus nodules" and a cross section of an artery with a thrombus.

PLATE XII

FIGURE 35. Skin excision, eleventh day. A capillary of the skin with hemorrhage. There is a mural thrombus and discontinuity of the endothelium. 550 diameters.

FIGURE 36. Skin. Autopsy 37, death on tenth day. A small artery of the subcutaneous fat is shown with thrombosis and perivascular infiltration.

PLATE XIII

FIGURE 37. Artery of skin. Autopsy 32, death on fourteenth day. Figures 84, 85, and 86, plates XXXII and XXXIII show individual endothelial cells from this thrombus at high power. Approximately 600 diameters.

FIGURE 38. Artery of testis. Autopsy 32, death on fourteenth day. A mural thrombus with necrosis of the vessel wall and perivascular reaction. Approximately 600 diameters.

PLATE XIV ·

FIGURE 39. Arteriole of skin, excised on seventh day of typhus. There is shown an early mural thrombus covered with endothelium, and an early proliferative perivascular lesion or "typhus nodule." 800 diameters.

PLATE XV

FIGURE 40. Arteriole of skin. Autopsy 15, death in second week, probably early. Shows attached mural thrombi composed almost wholly of phagocytic endothelial cells. There is an early proliferative perivascular reaction. 400 diameters.

PLATE XVI

FIGURE 41. Myocardium. Autopsy 11, death on seventh day. To show diffuse and focal lesions. Approximately 125 diameters. (Compare with drawing, Plate XVII, figure 43.)

FIGURE 42. Skeletal muscle. Autopsy 37, death on tenth day. Shows diffuse infiltration, one typhus "nodule," and perivascular infiltration. Approximately 125 diameters.

PLATE XVII

FIGURE 43. Myocardium. Autopsy 9, death on ninth day. Shows infiltrations between muscle fibers and a minute necrosis of the muscle. 825 diameters.

FIGURE 44. Adrenal gland cortex showing a type of lesions common in many infectious diseases as well as typhus. Autopsy 1, death on tenth day. 825 diameters.

PLATE XVIII

FIGURES 45 and 46. Photomicrographs of the Ammon's horn and cerebral cortex respectively of guinea-pig 219, a passage virus animal (see chart 2). This guinea-pig became paralyzed the day following its return to normal temperature, which was seventeen days after the inoculation. The figures illustrate the size and distribution of the proliferative lesions in guinea-pigs. Approximately 100 diameters.

PLATE XIX

FIGURE 47. A higher power photomicrograph of one of the lesions shown in Plate XVIII, figure 46, guinea-pig 219. A central capillary is shown. Polymorphonuclear leucocytes are present although the stage of the disease was late and the temperature normal. Approximately 500 diameters.

FIGURE 48. Cerebral cortex. Autopsy 21, death on fifteenth day. To show two compact proliferative lesions and perivascular infiltration. See high power photomicrograph, Plate XXI, figure 52, and drawing Plate XXV, figure 60, for details of compact lesion in lower left quadrant. Approximately 100 diameters.

PLATE XX

FIGURE 49. Olivary nucleus, medulla. Autopsy 20, death on twentieth day. Two compact proliferative lesions in one 16 mm. objective field. Approximately 150 diameters.

FIGURE 50. Higher power photomicrograph of one of the lesions in Figure 49. The peripheral condensation of cells, neuroglia, is a feature of late lesions. Approximately 600 diameters.

PLATE XXI

FIGURE 51. Cerebellum. Autopsy 16, death on fourteenth day. A compact proliferative lesion showing relation to a capillary. Compare with drawing, Plate XXIII, figure 56. Approximately 500 diameters.

FIGURE 52. Compact proliferative lesion in central cortex. Autopsy 21, death on fourteenth day. See low power photomicrograph, Plate XIX, figure 48 and drawing, Plate XXV, figure 60. Approximately 350 diameters.

PLATE XXII

FIGURE 53. An early cerebral lesion in guinea-pig 19 ten days after inoculation with viscera of louse 238. See protocol, page 56. The drawing shows thrombosis, proliferation of endothelium, and phagocytosis, and early reaction of the perivascular neuroglia. Note the polymorphonuclear leucocytes. Approximately 1,000 diameters.

FIGURE 54. An early proliferative lesion of the compact type in the superficial layer of the cerebral cortex. Human. Autopsy 29, death on fourteenth day. A mitotic figure, probably of the endothelium, is present. The lesion is situated at the branching of a precapillary which shows proliferation and discontinuity of the endothelium. 550 diameters.

PLATE XXIII

FIGURE 55. A loose textured proliferative lesion in the superficial layer of the cerebral cortex. The cells are almost exclusively neuroglia cells. Rod cells and cells with sausage-shaped nuclei are in the adjacent tissue. Zeiss 4 mm. apo. 4 comp. ocular. 400 diameters.

FIGURE 56. A proliferative lesion of the compact type in the molecular layer of the cerebellum, showing relation to a capillary. Autopsy 16, death on fourteenth day. Compare with photomicrograph, Plate XXI, figure 52. 400 diameters.

PLATE XXIV

FIGURE 57. A small hemorrhage in the superficial layer of the cerebral cortex. Autopsy 8, death on seventh day. The red blood cells have been taken up by macrophages (endothelial leucocytes). The other cells are possibly of neuroglia origin. A rod (Stäbchen) cell is shown on the right. 600 diameters.

FIGURE 58. A loose textured proliferative lesion in the molecular layer of the cerebellum. Autopsy 2, death on the fifteenth day. This lesion represents the nearest approach to the strauchartiges lesion described by Spielmeyer that we have encountered. 240 diameters.

PLATE XXV

FIGURE 59. A small mural thrombus covered with endothelium on the endocardium. From a case with thrombosis of the internal carotid artery. Autopsy 18, death on twelfth day. 240 diameters.

FIGURE 60. A compact proliferative lesion in the cerebral cortex. Autopsy 21, death on fifteenth day. This is an illustration of a typical typhus lesion and demonstrates the proliferative character of the lesion and absence of necrosis. At the periphery are numerous elongated neuroglia cells with processes. 400 diameters.

PLATE XXVI

FIGURES 61, 62, and 63. Capillaries and precapillaries from two early cases of Mexican typhus showing rickettsia in the swollen endothelium. (See Wolbach and Todd, 1920, 1.) Compare Figure 62 with photomicrograph in Plate XXXIII, figure 84.

FIGURE 64. Louse Box 53, Louse 2. *Rickettsia prowazeki* and *Rickettsia pediculi*.
Section. The greatly swollen and vacuolated epithelial cells of the midgut are filled with enormous numbers of *Rickettsia prowazeki*; these usually occur as palely staining granules, either singly or in pairs. Among them are larger granules which stain more deeply and are seen more clearly. The lumen of the gut is heavily infected with larger, more deeply staining rickettsia. These, like the organisms which thickly cover the cuticular border of the gut cells, are *Rickettsia pediculi*. They occur as granules either singly or in pairs. 1,800 diameters.

PLATE XXVII

FIGURE 65. A venule of the skin showing a compact cluster of small paired rickettsia. The vessel shows an early lesion, the endothelium is swollen and has taken up red corpuscles. The intima is invaded by leucocytes. There is also a perivascular accumulation of endothelial leucocytes. Compare with the photomicrograph in Plate XXX, figure 75. 1,800 diameters.

FIGURE 66. Large paired rickettsia in endothelial cells *in situ* in an arteriole of the skin cut longitudinally. Autopsy 1, death on tenth day. Compare with the photomicrograph in Plate XXXII, figure 81. 1,800 diameters.

FIGURE 67. Artery of the skin, a portion of the same vessel shown in Plate XXVIII, figure 68. A swollen phagocytic endothelial cell *in situ* is filled with the small forms of rickettsia. Autopsy 1, death on tenth day. Compare with the photomicrograph in Plate XXXII, figure 80. 1,800 diameters.

PLATE XXVIII

FIGURE 68. Large forms of rickettsia in an endothelial cell of an artery of the skin. Other parts of the same section through this vessel show mural thrombi and the endothelial cell illustrated in Plate XXVII, figure 67. Autopsy 1, death on tenth day. 1,200 diameters.

FIGURE 69. Early vascular lesion in cerebral cortex of guinea-pig 46 (see protocol, page 105) inoculated with viscera of Louse 2, Box 38. A precapillary with rickettsia in endothelial cells, scattered and in a clump indicated by the arrow. Animal killed on third day of temperature. Compare with photomicrograph, Plate XXXI, figure 78. 1,800 diameters.

PLATE XXIX

FIGURE 70. Arteriole of skin, showing swollen degenerated endothelial cells and an early fibrin mural thrombus. Two endothelial cells contain rickettsia. There is an early perivascular reaction (endothelial leucocytes). Autopsy 29, death on fourteenth day. 1,800 diameters.

FIGURE 71. Precapillary arteriole of skin, showing paired large rickettsia in the swollen endothelium. Autopsy 14, death on tenth day. Compare with photomicrograph, Plate XXXII, figure 82. 1,200 diameters.

FIGURE 72. Arteriole of skin, showing early typhus lesion, degeneration of the endothelium and beginning mural thrombus. The swollen endothelium contains many small rickettsia bodies. Autopsy 32, death on fourteenth day. 1,200 diameters.

FIGURE 73. Capillary of skin with five pairs of large rickettsia in the swollen epithelium. A mast cell lies close to this capillary and is illustrated for the comparison of mast cell granules and rickettsia. The former are larger and stain more deeply and with a different coloration. Autopsy 3, death on ninth day. 1,800 diameters.

FIGURE 74. Two mast cells lying close to capillaries of the skin (corium). Illustrated to show size and coloration of the granules and length of the cytoplasmic processes of the mast cells. Skin excised on eighth day of typhus. 1,200 diameters.

PLATE XXX

FIGURE 75. Photomicrograph of a part of the field drawn in Plate XXVII, figure 65. To show clump of rickettsia. 2,000 diameters.

FIGURE 76. Capillary, cerebral cortex of guinea-pig 14 inoculated with viscera of Louse W 223, Box 19. (See protocol, page 70.) To show rickettsia in endothelial cells. See low power photomicrograph in Plate XXXI, figure 77. 2,000 diameters.

PLATE XXXI

FIGURE 77. Cerebral cortex of guinea-pig 14 inoculated with the viscera of Louse W 223, Box 19. (See protocol, page 70.) Photomicrograph to show early vascular lesion. Approximately 600 diameters.

FIGURE 78. Photomicrograph of field shown in drawing, Plate XXVIII, figure 69. To show rickettsia in endothelial cells in an early vascular lesion in brain of a guinea-pig. 2,000 diameters.

PLATE XXXII

FIGURE 79. Photomicrograph of the field drawn in Plate XXVIII, figure 68. To show rickettsia in an endothelial cell. Artery of skin. Autopsy 1, death on tenth day. 2,000 diameters.

FIGURE 80. Photomicrograph of the field drawn in Plate XXVII, figure 67. To show minute forms of rickettsia in an endothelial cell *in situ*. Autopsy 1, death on tenth day. 2,000 diameters.

FIGURE 81. Photomicrograph of upper part of the field drawn in Plate XXVII, figure 66. To show rickettsia in an endothelial cell. These are the large paired rickettsia. Autopsy 1, death on tenth day.

FIGURE 82. Photomicrograph of arteriole drawn in Plate XXIX, figure 71. The arrows point to rickettsia. Note the pair of large forms. Autopsy 14, death on tenth day. 1,500 diameters.

FIGURE 83. Photomicrograph. Artery of skin. Autopsy 32, death on fourteenth day. Rickettsia in endothelial cell. This cell lies at the base of a mural thrombus shown at low power in Plate XIII, figure 37. 2,000 diameters.

FIGURE 84. Photomicrograph of a part of the field drawn in Plate XXVI, figure 62. Skin. Mexican typhus. Rickettsia in endothelium in precapillary arteriole. 2,000 diameters.

PLATE XXXIII

FIGURES 85 and 86. Two endothelial cells with rickettsia from the surface of the thrombus shown at low power in Plate XIII, figure 37. Autopsy 32, death on fourteenth day. Artery of skin. 2,000 diameters.

FIGURE 87. Artery of skin. Autopsy 7, death on ninth day. The arrow points to a clump of rickettsia bodies. This artery showed very slight lesions. 2,000 diameters.

PLATE XXXIV

FIGURE 88. Testis. Autopsy 29, death on fourteenth day. An artery of the tunica with a thrombus. The section passes longitudinally through several endothelial cells which in Figure 89 are shown at high power. 600 diameters approximately.

FIGURE 89. To show many rickettsia in the endothelial cells adjacent to the thrombus shown in Figure 88. There are both the large paired rickettsia and the small forms. 2,000 diameters.

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PLATE I

7a

7b　　　　7c

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PLATE II

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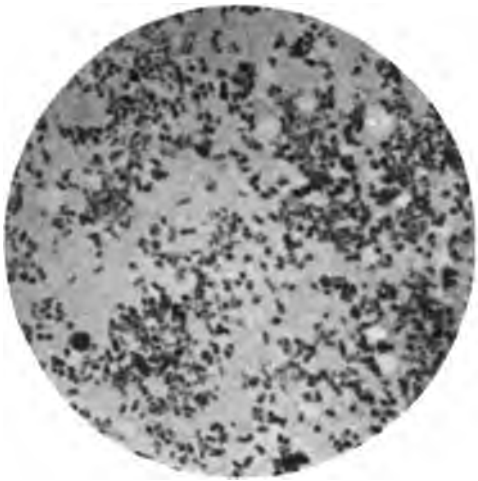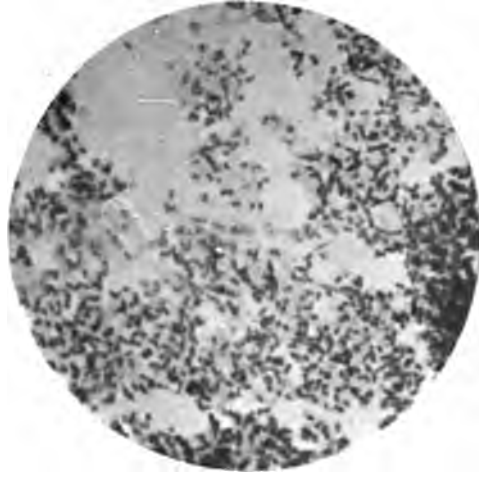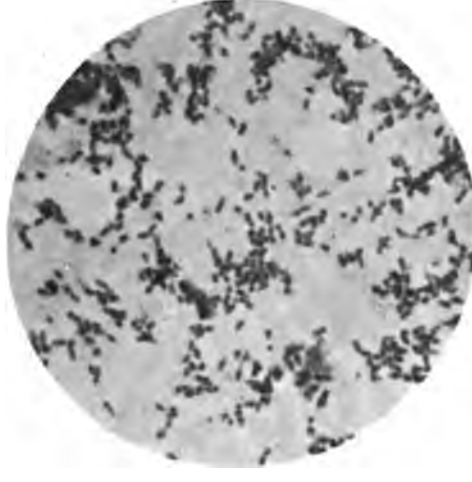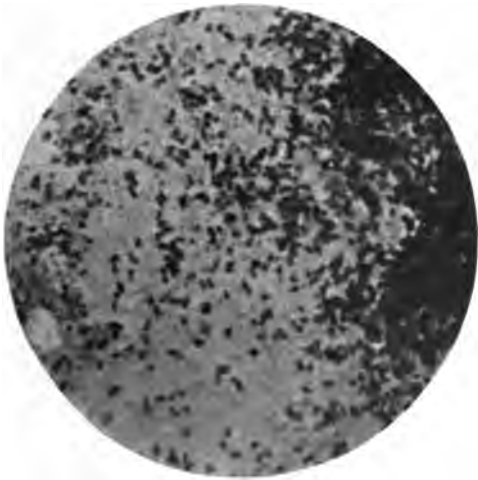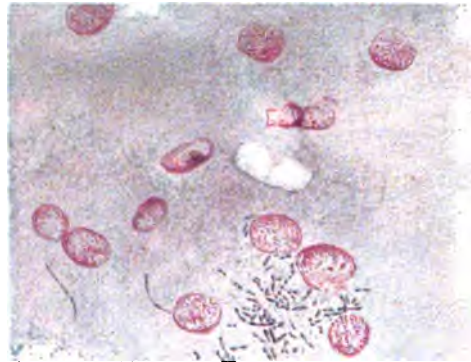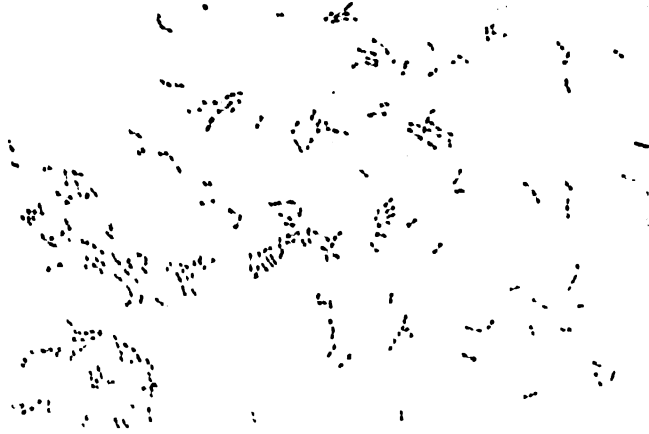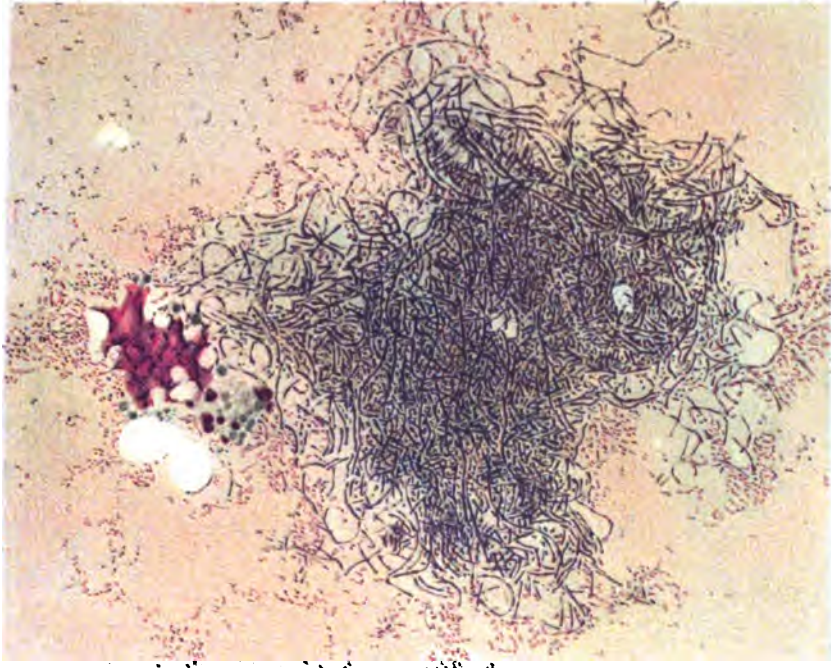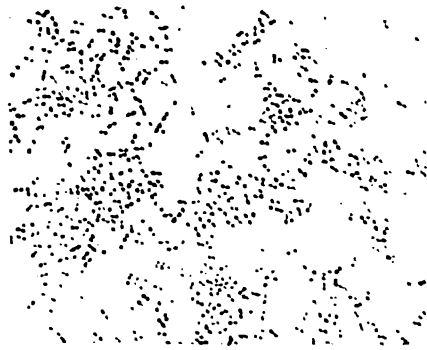
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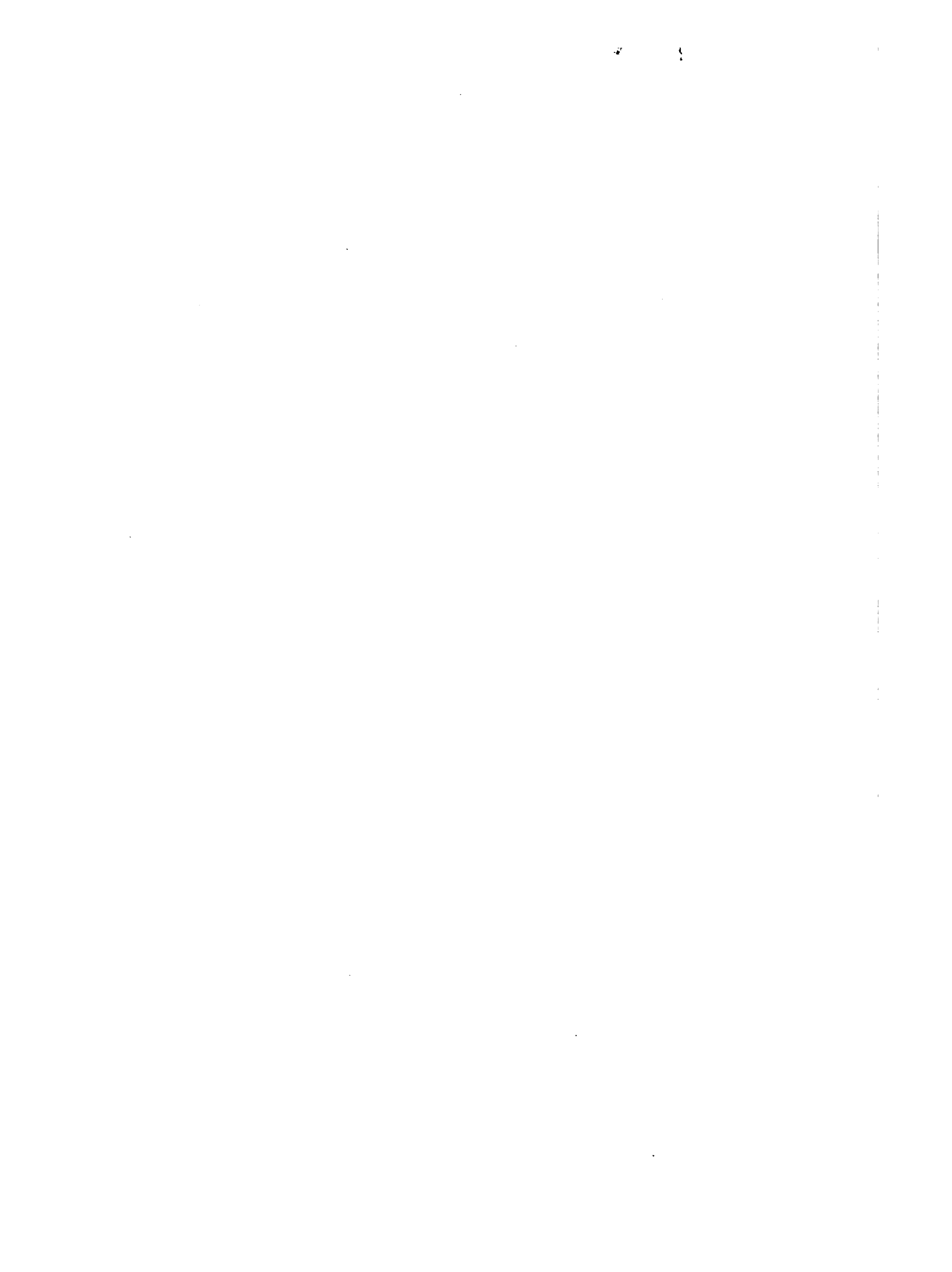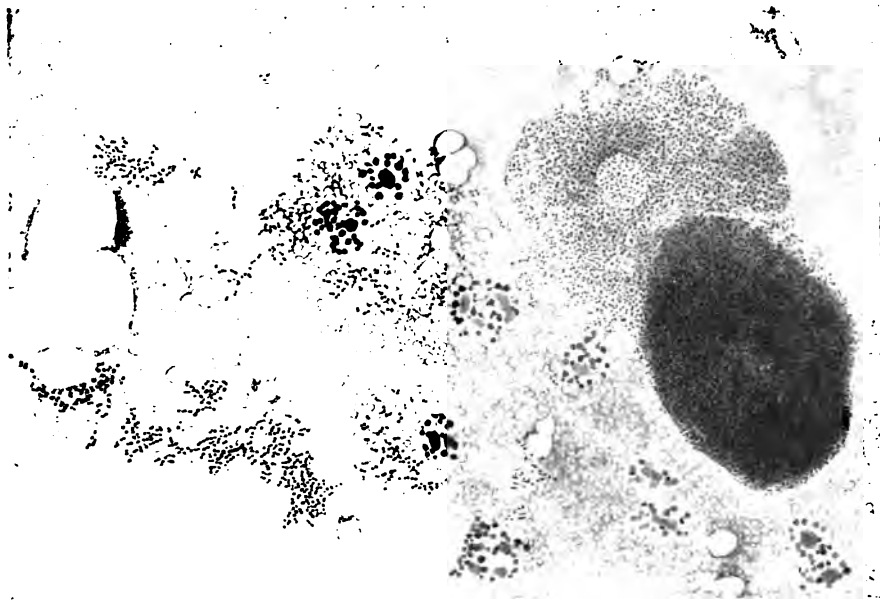
PLATE III

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PLATE IV
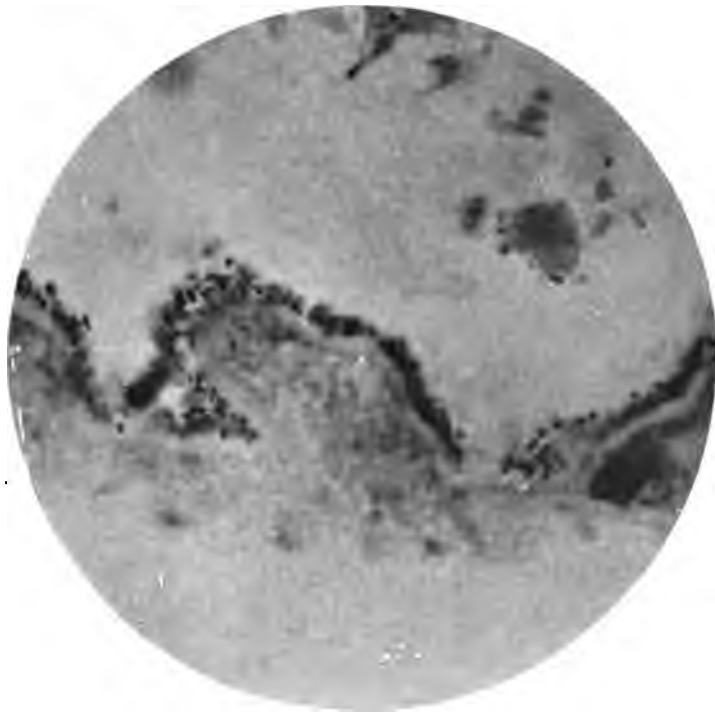
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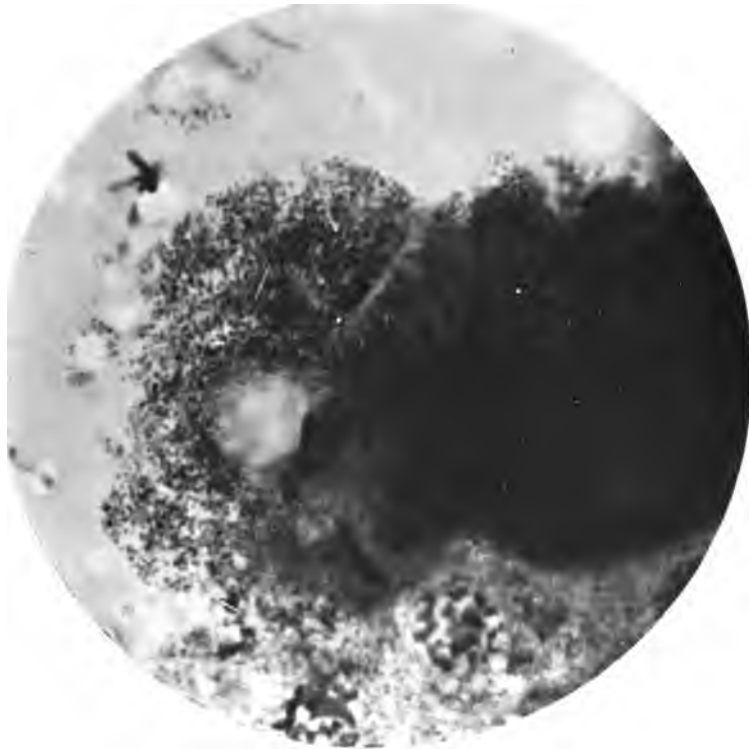
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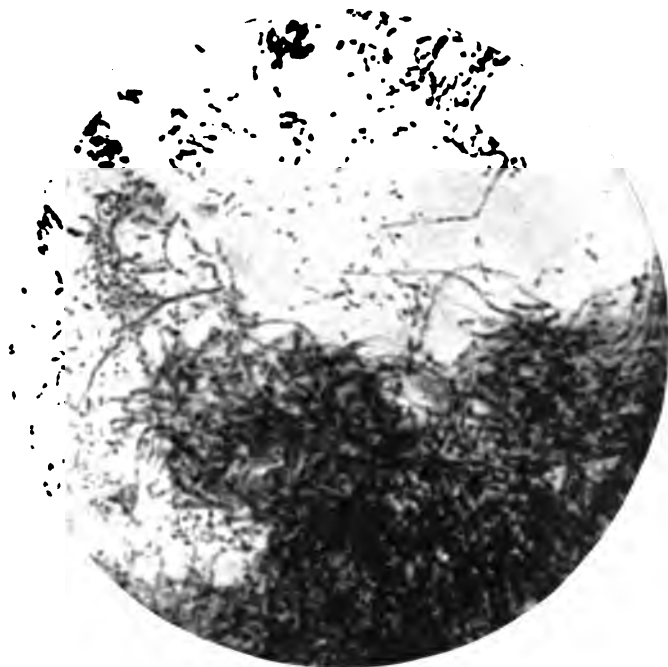
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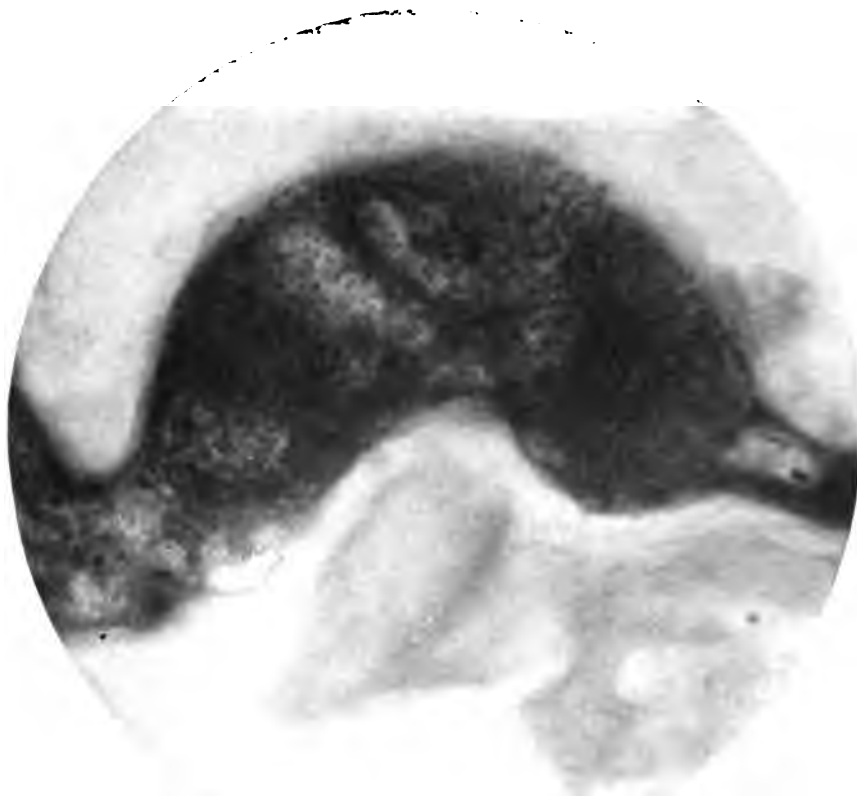
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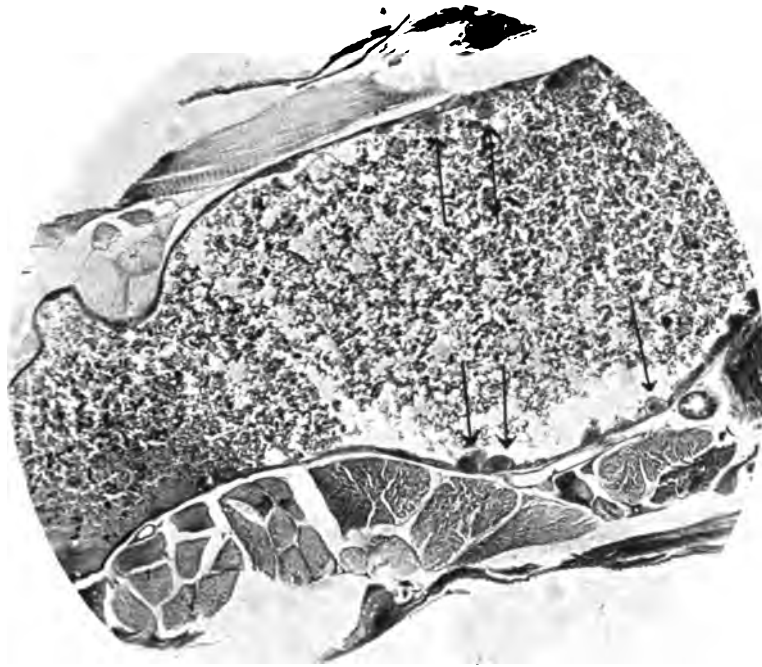
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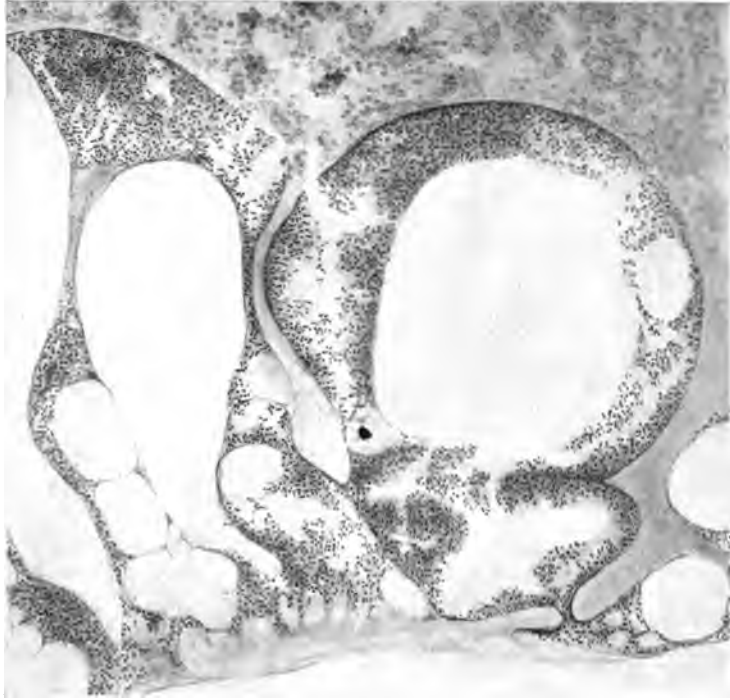
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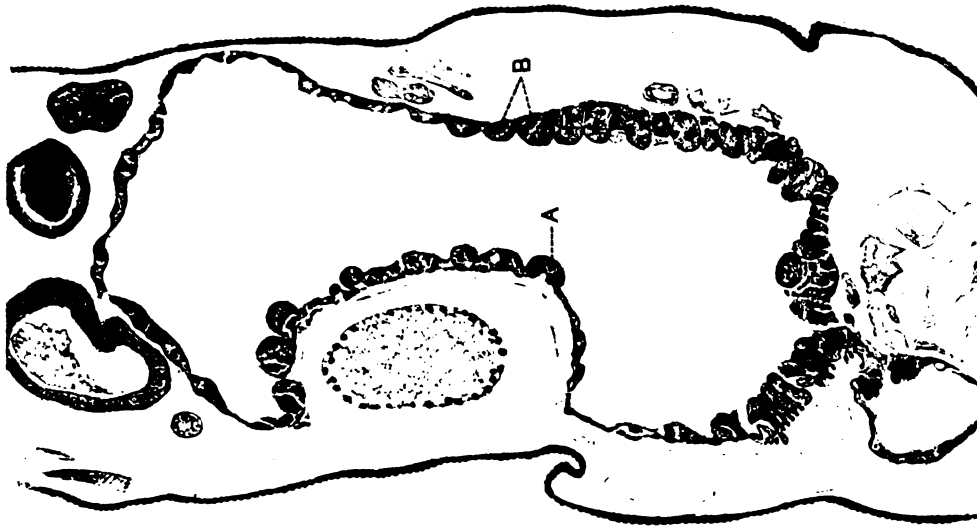
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Plate VIII

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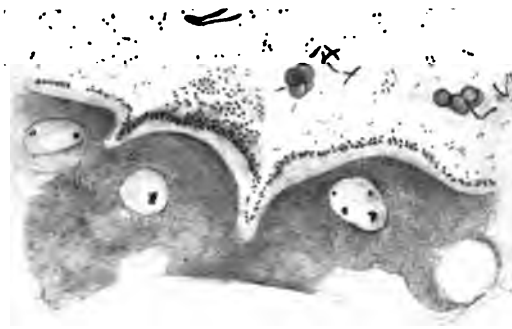
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PLATE IX

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PLATE X
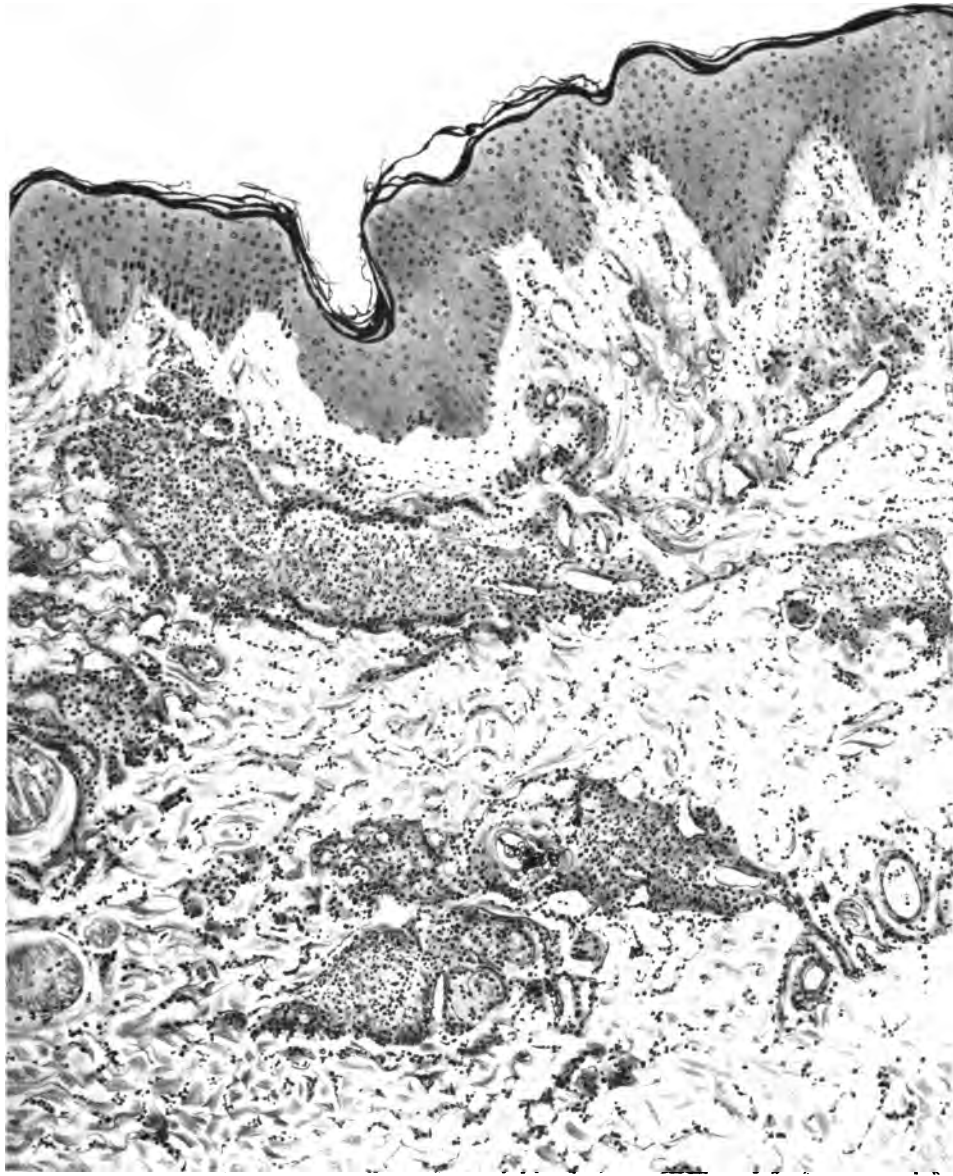
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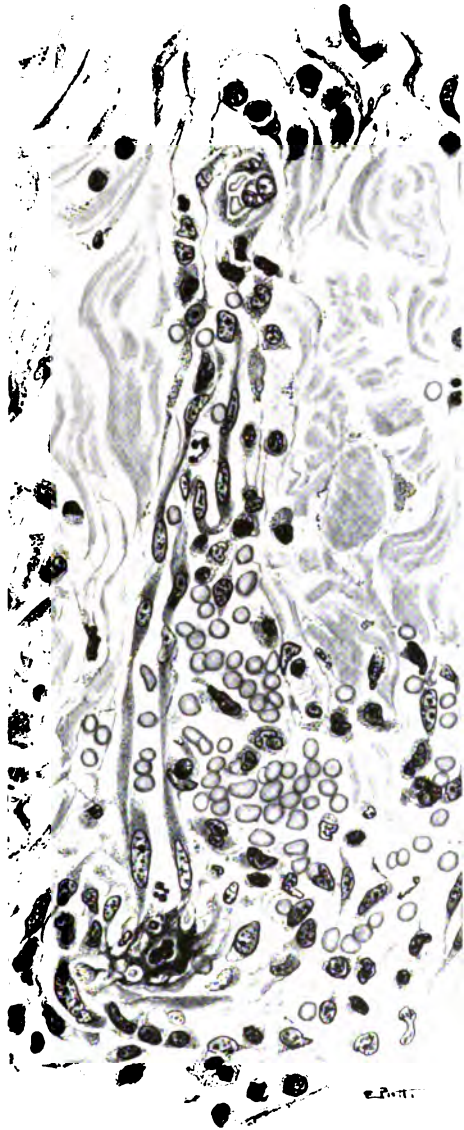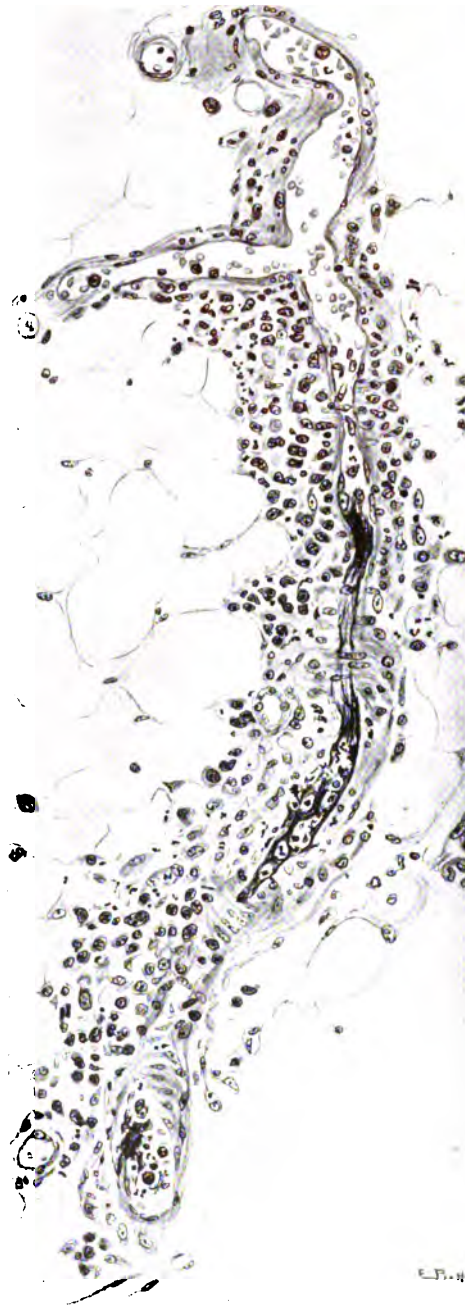
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Plate XII

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PLATE XIII

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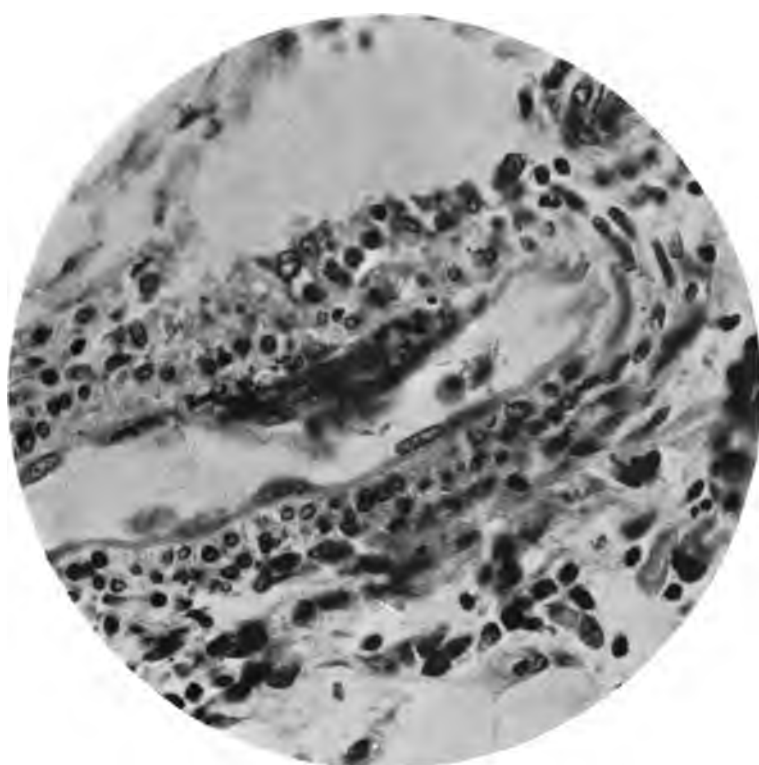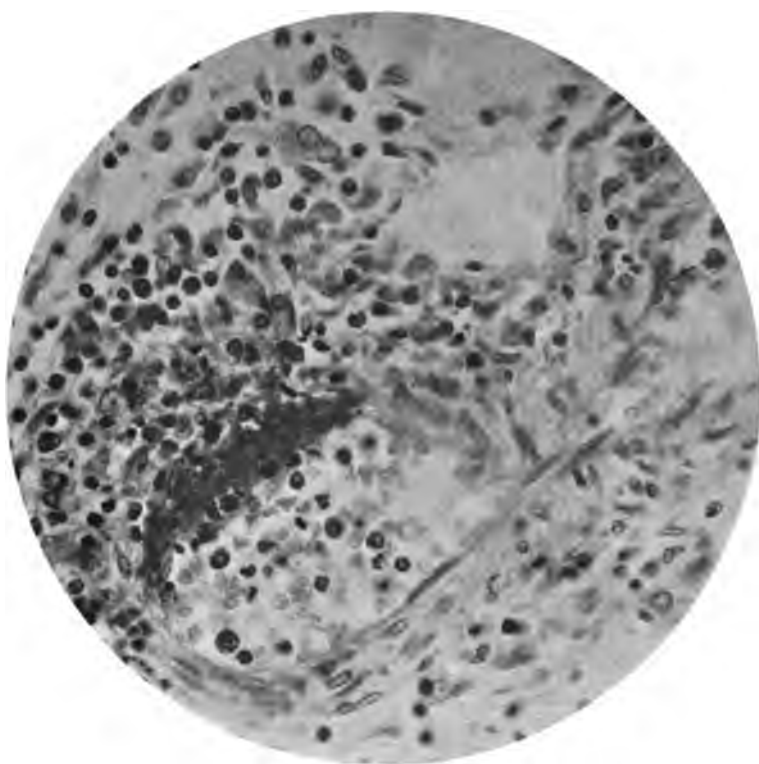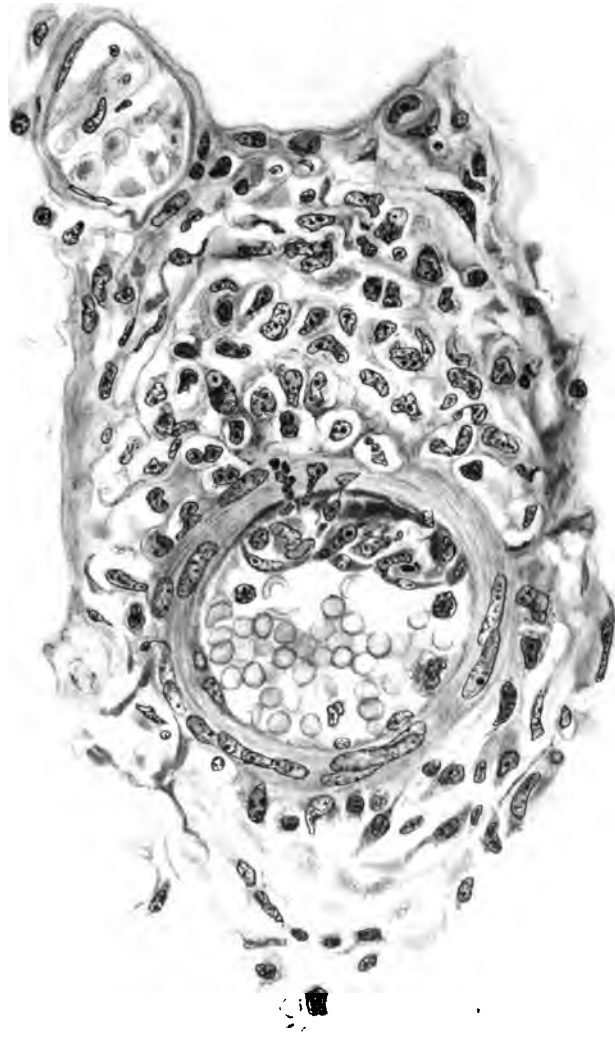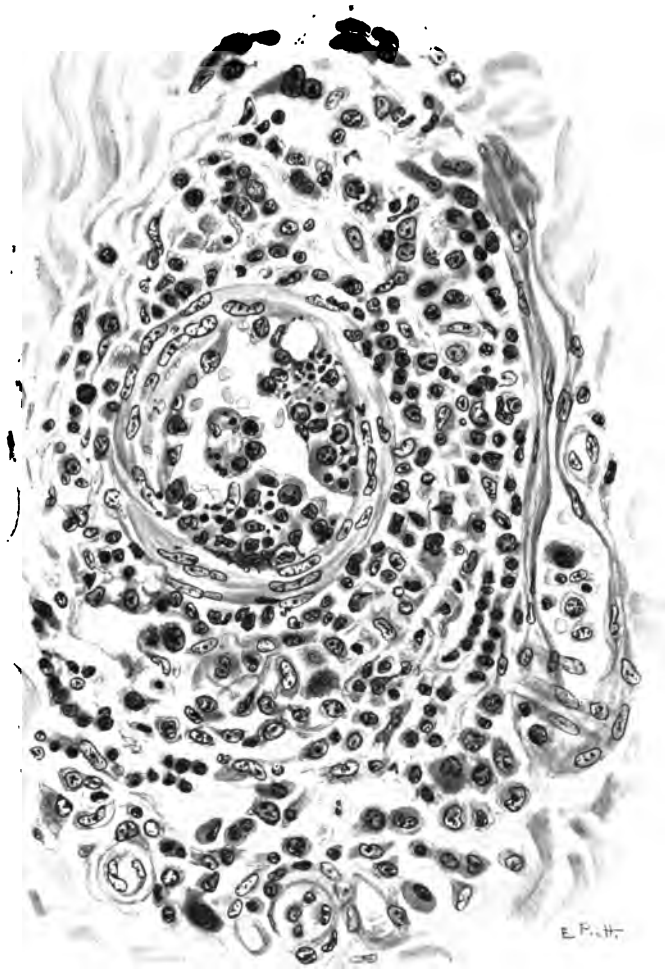
PLATE XIV

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PLATE XV

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Plate XVI

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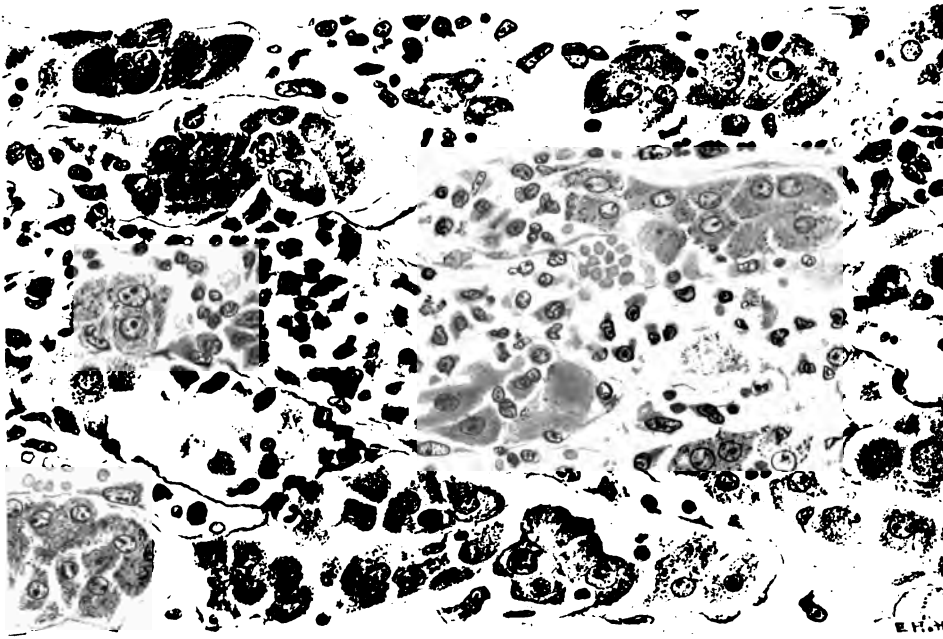
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PLATE XVII

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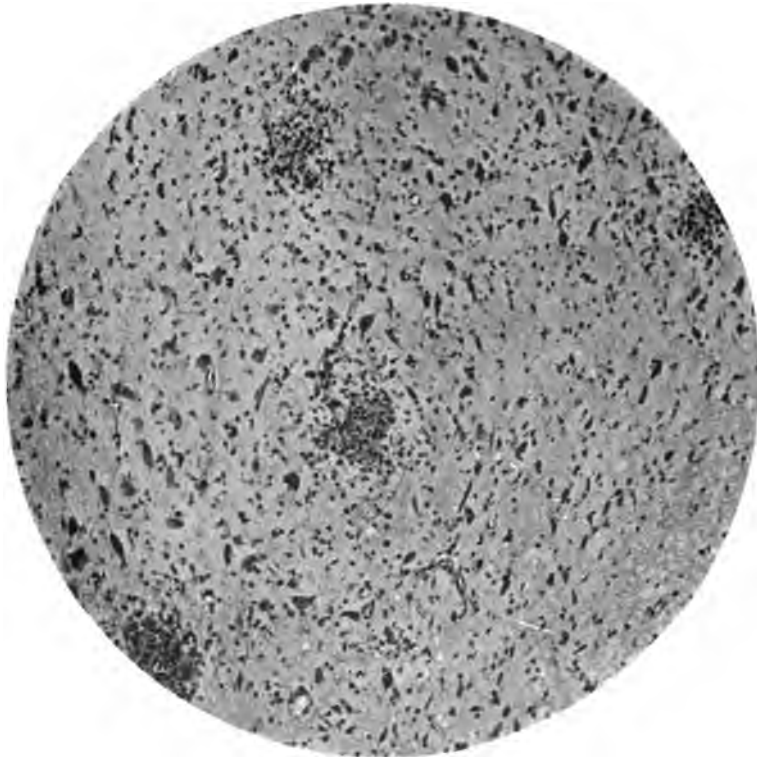
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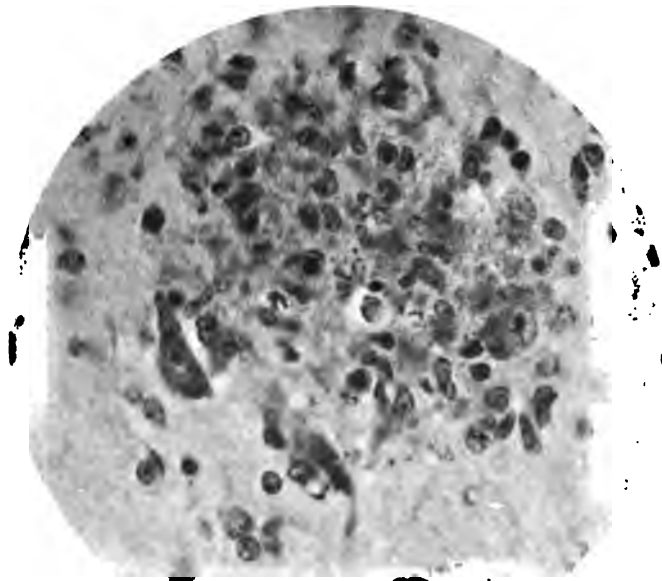
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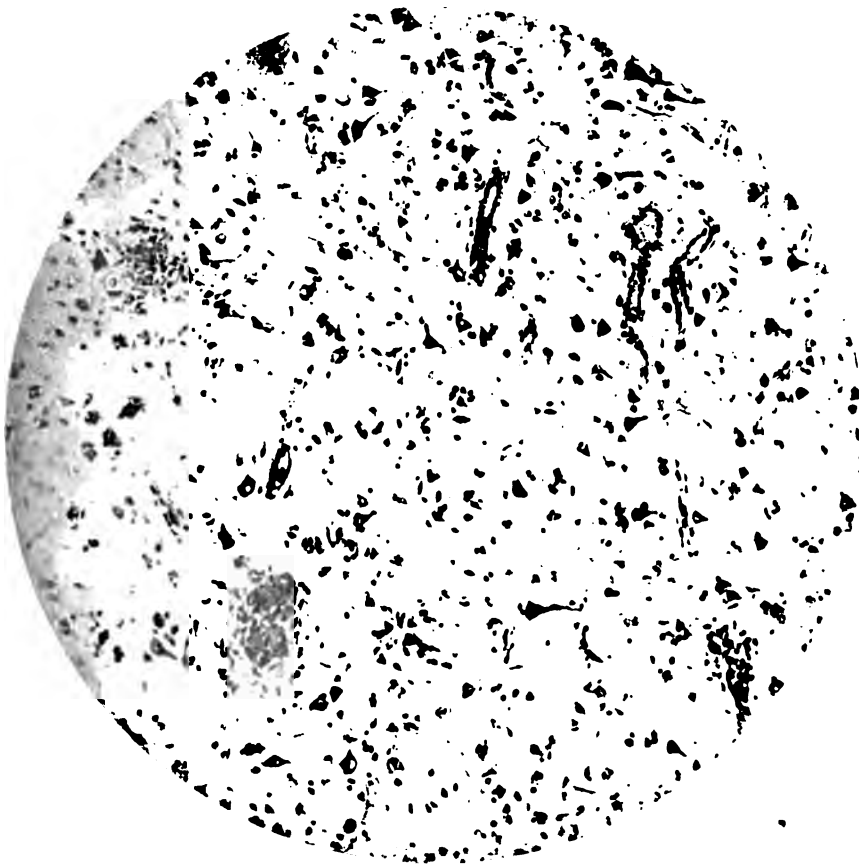
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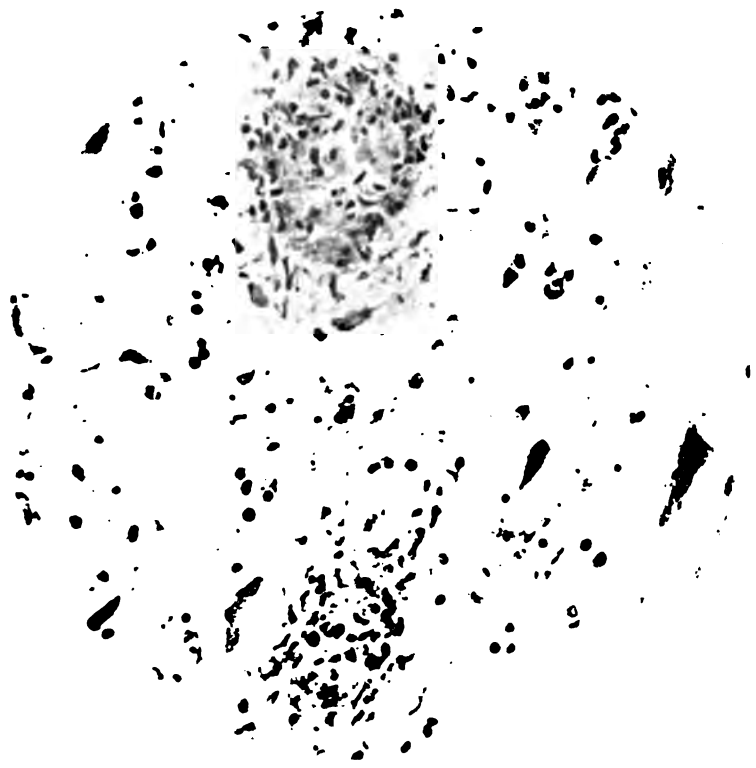
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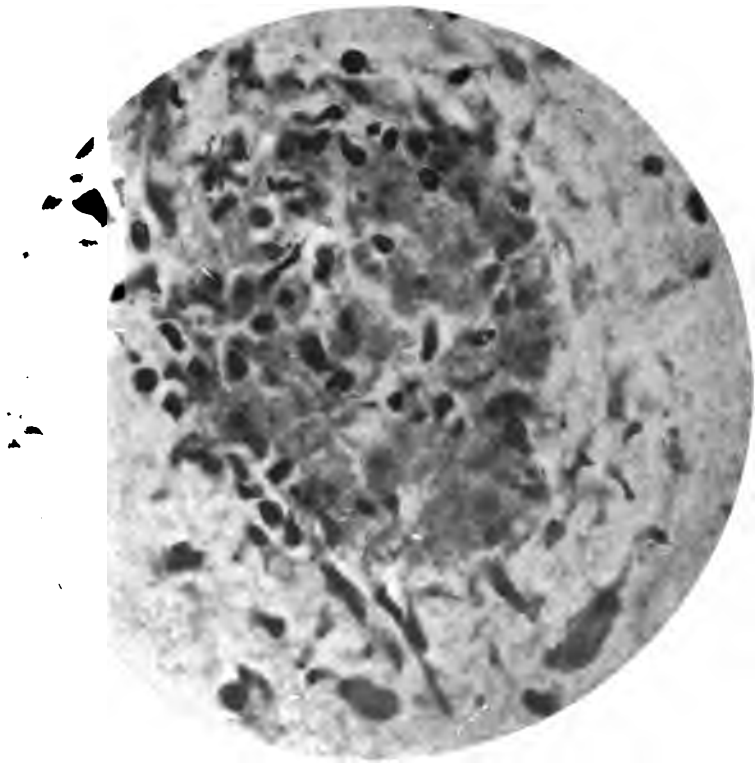
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PLATE XX
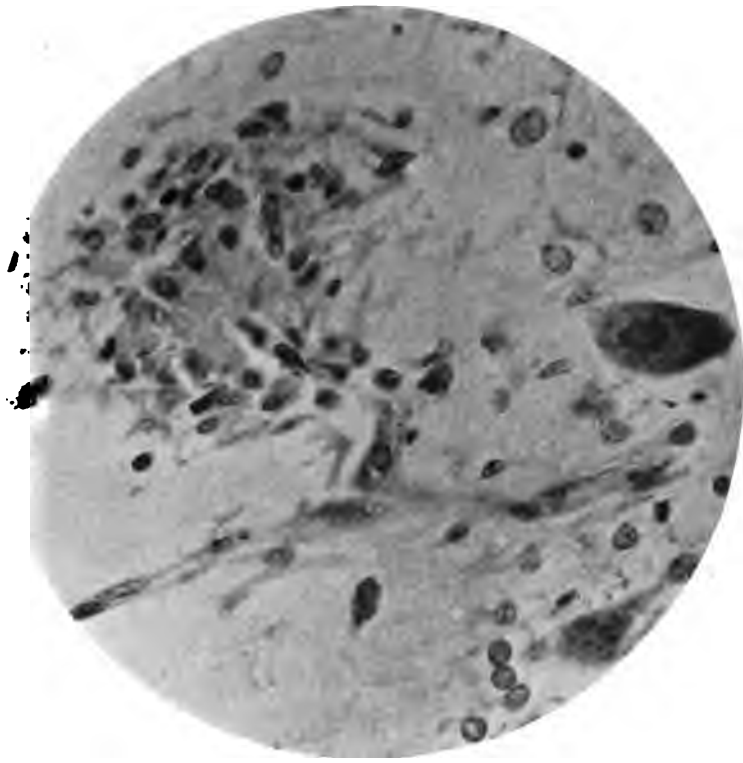
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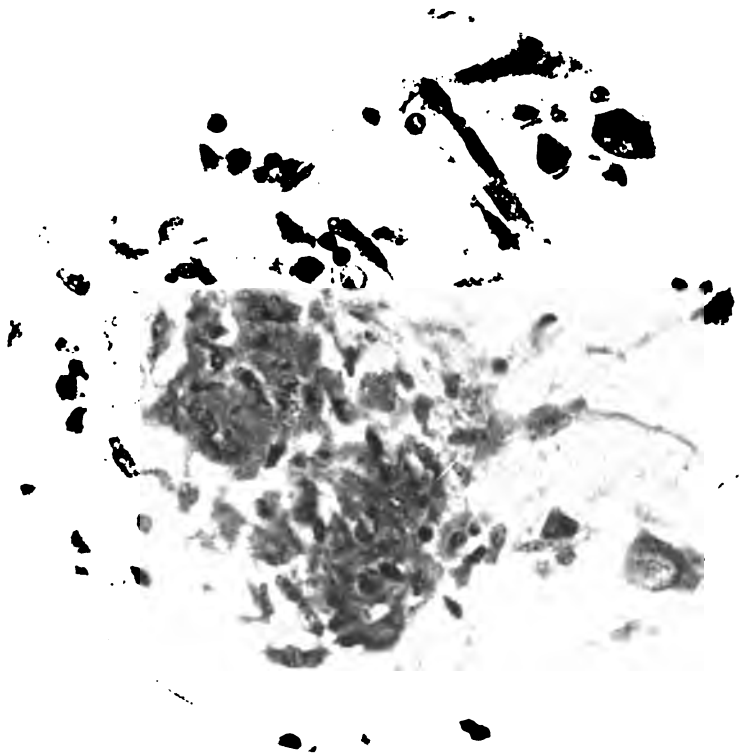
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Plate XXI
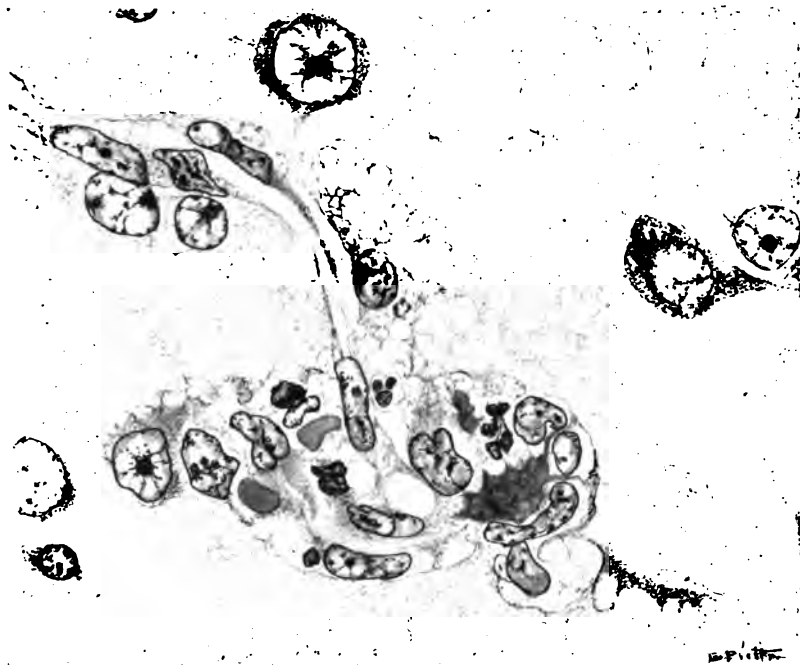
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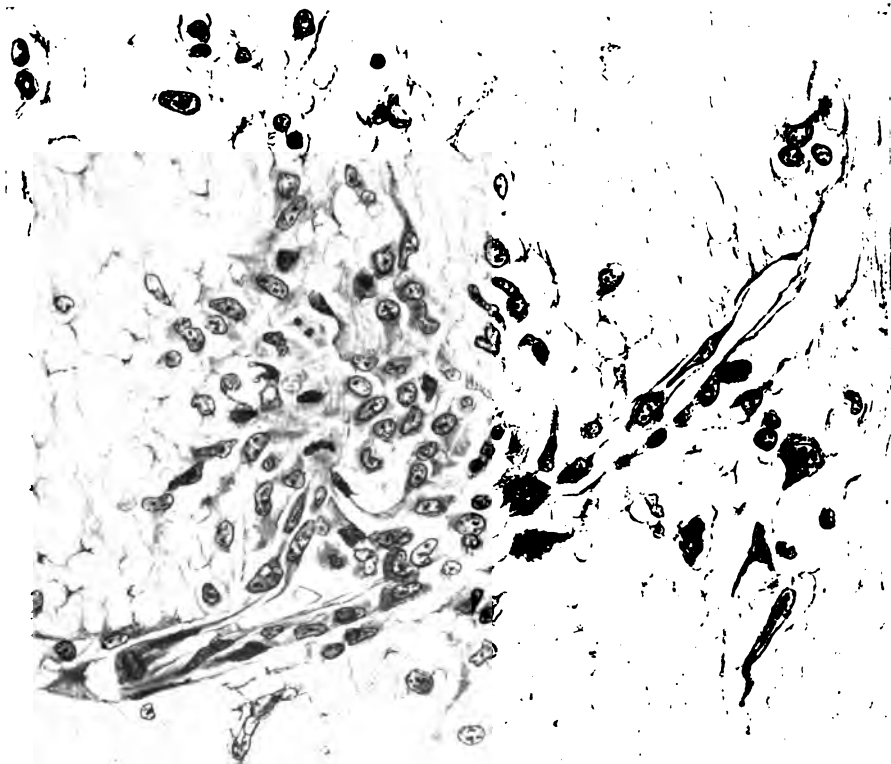
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PLATE XXII
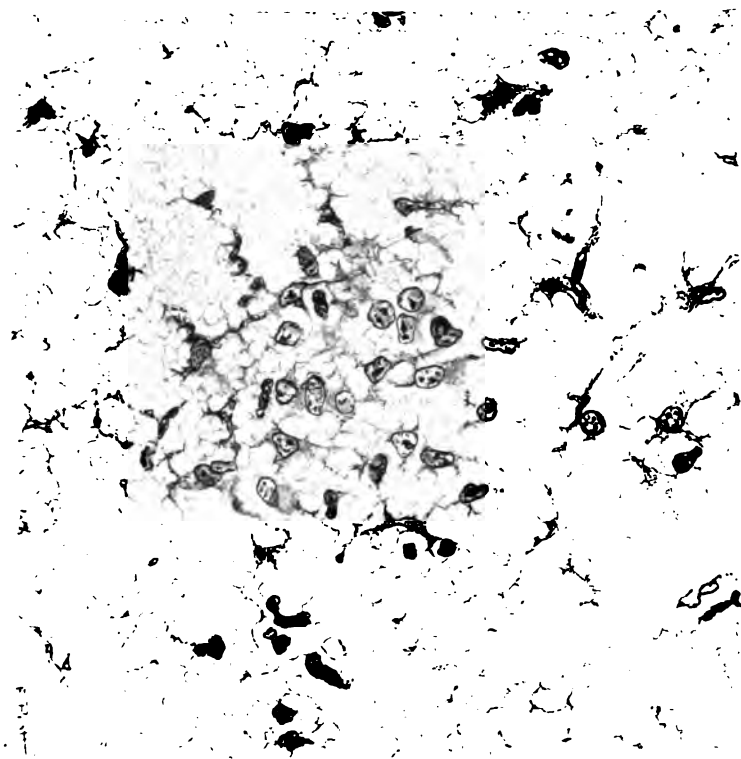
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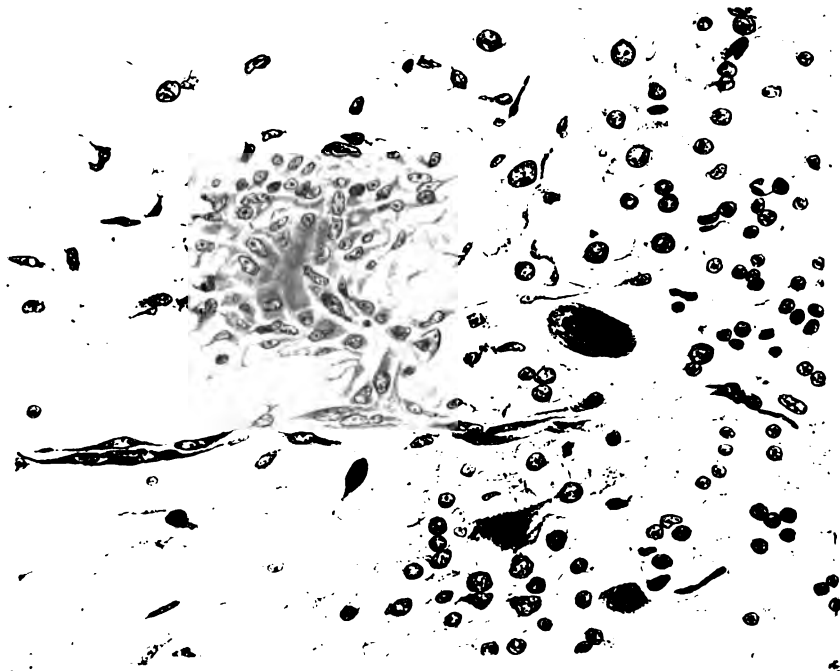
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Plate XXIII

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Plate XXIV

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PLATE XXV

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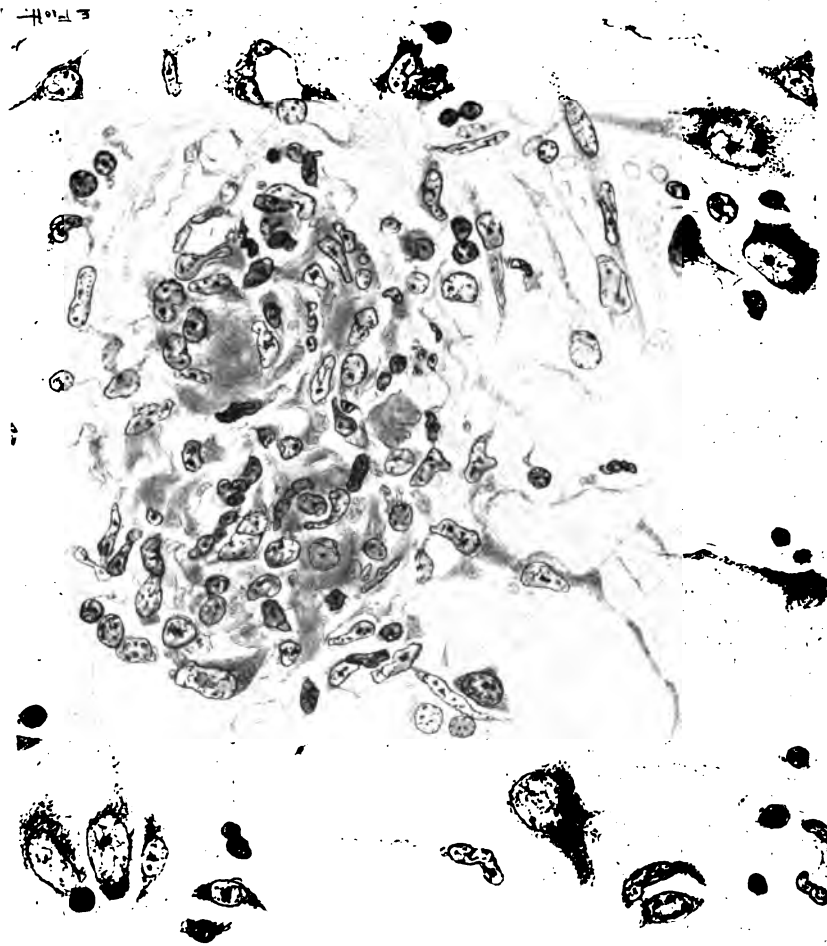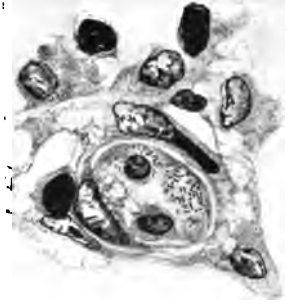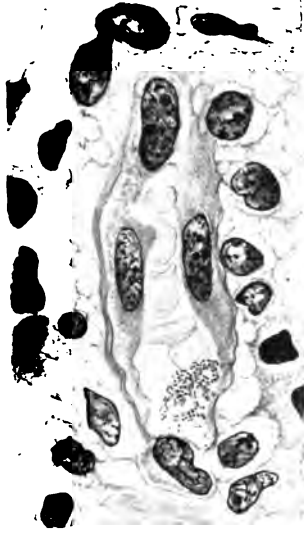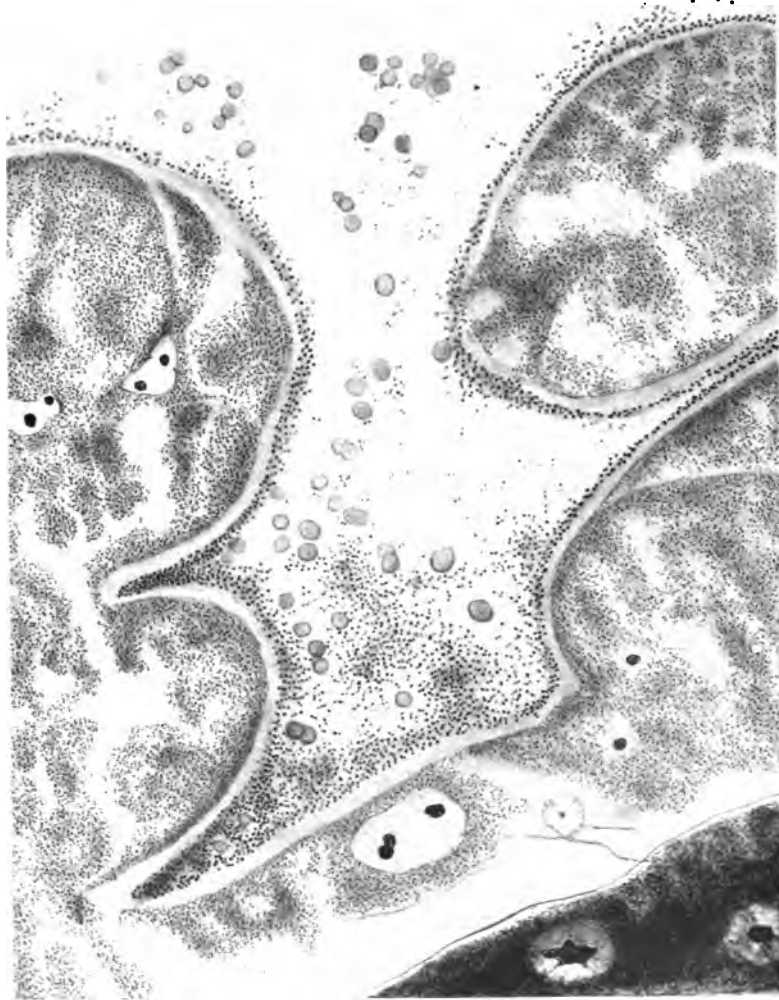
PLATE XXVI

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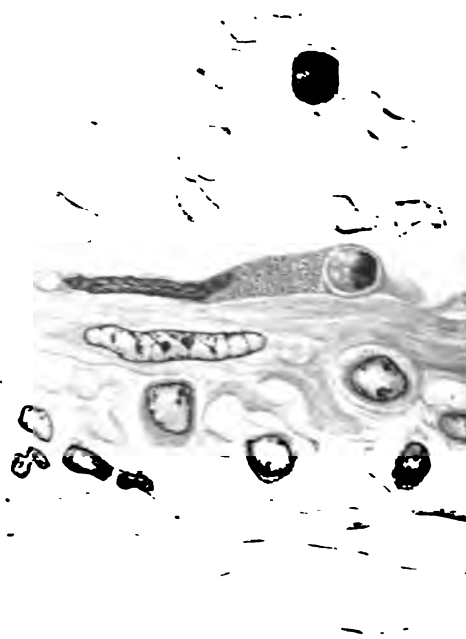
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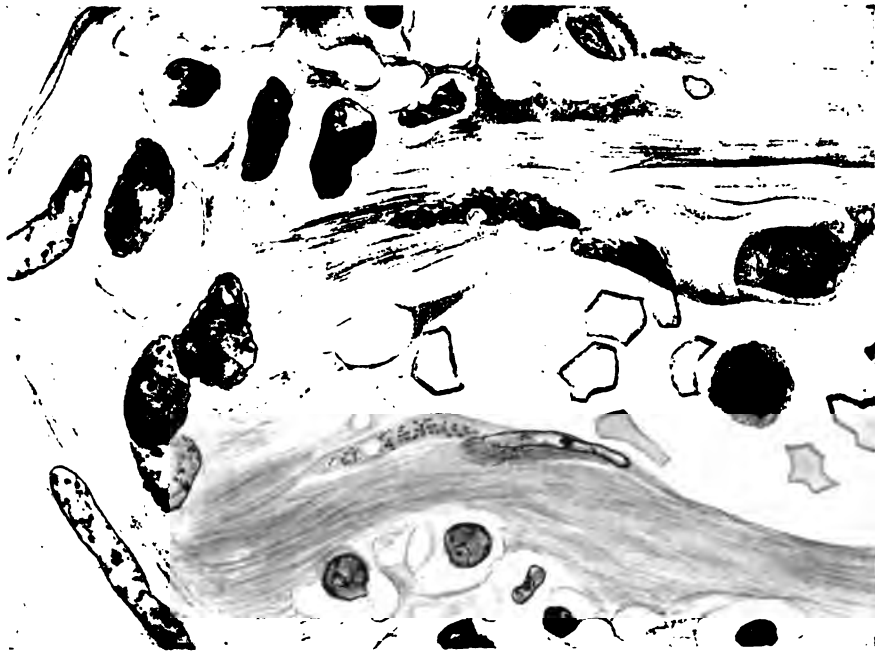
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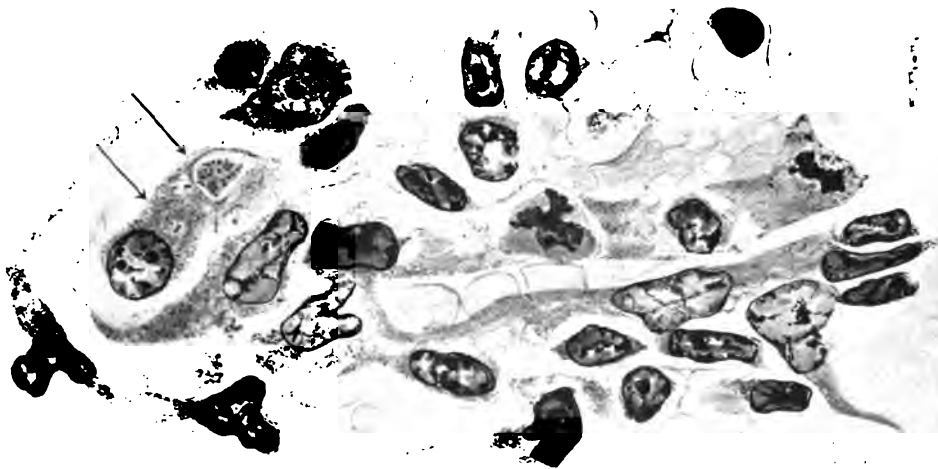
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Plate XXVIII

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PLATE XXIX
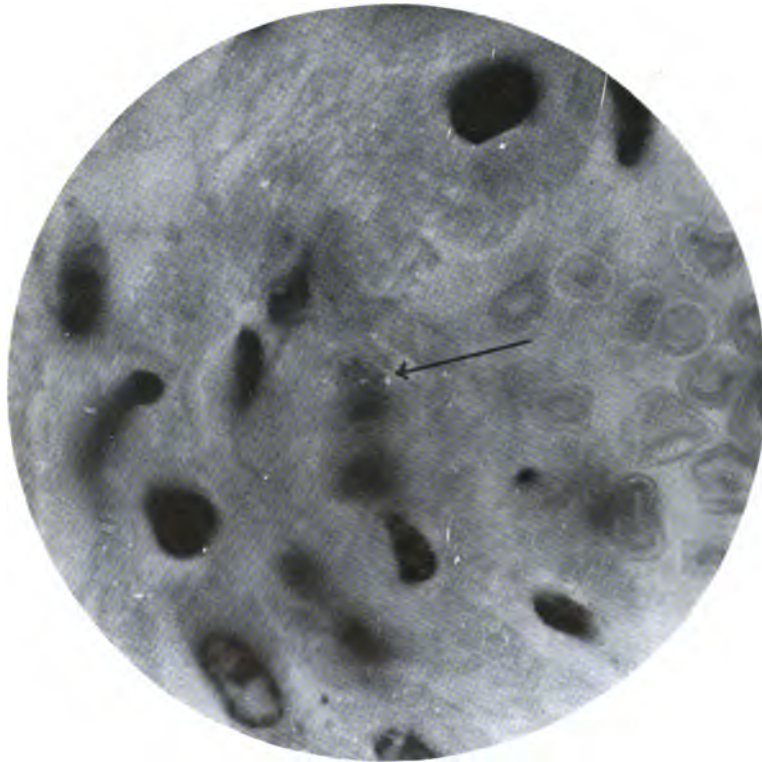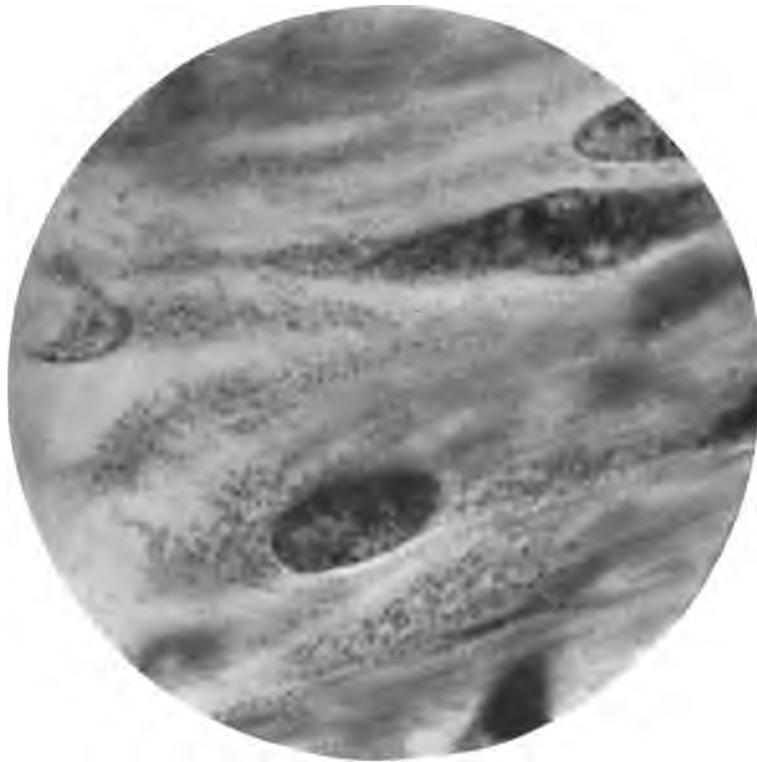
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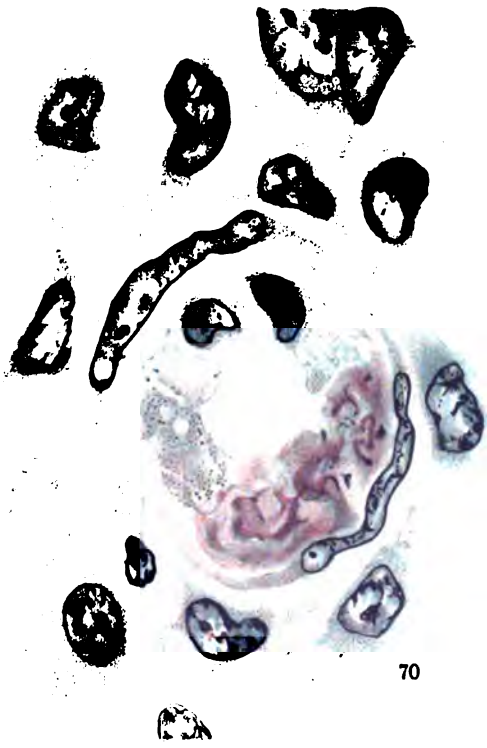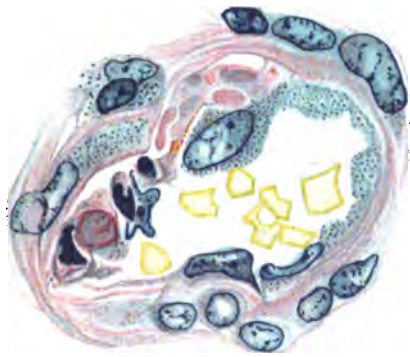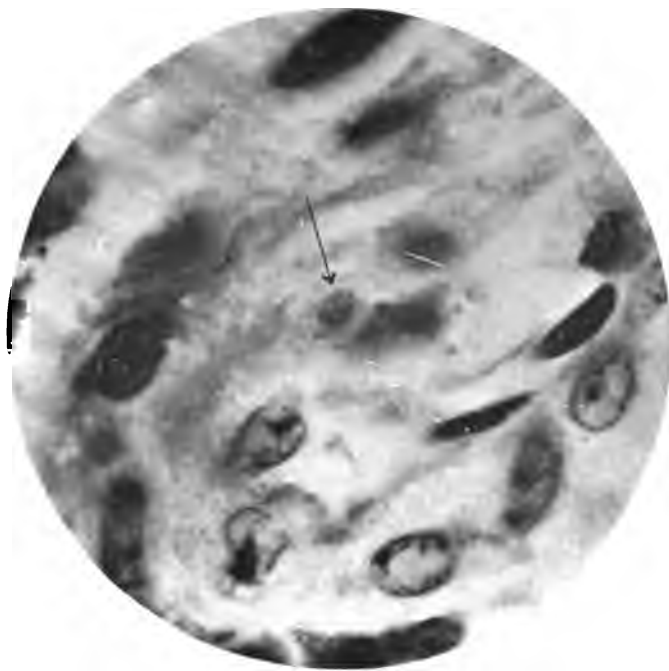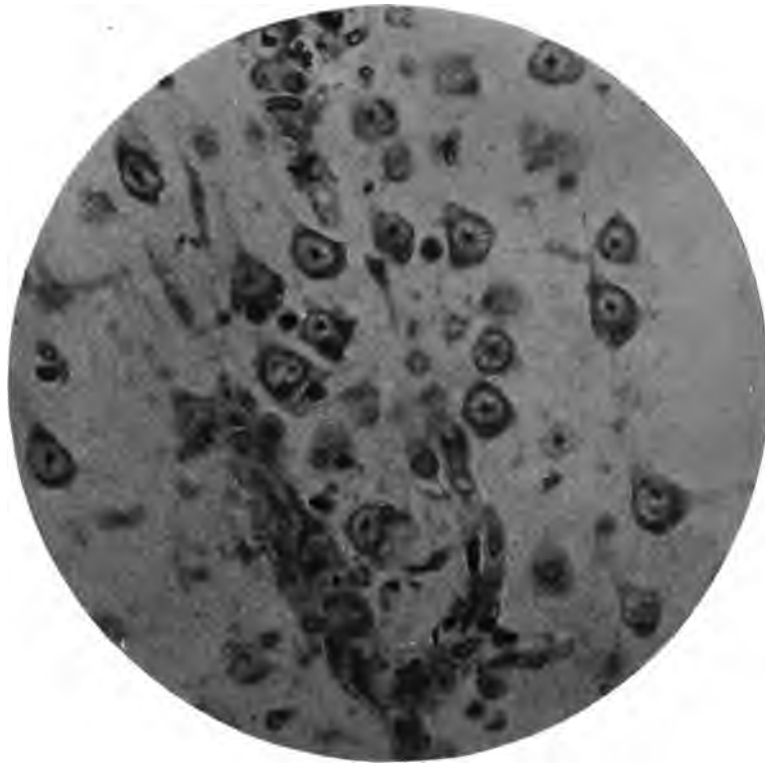
Plate XXX

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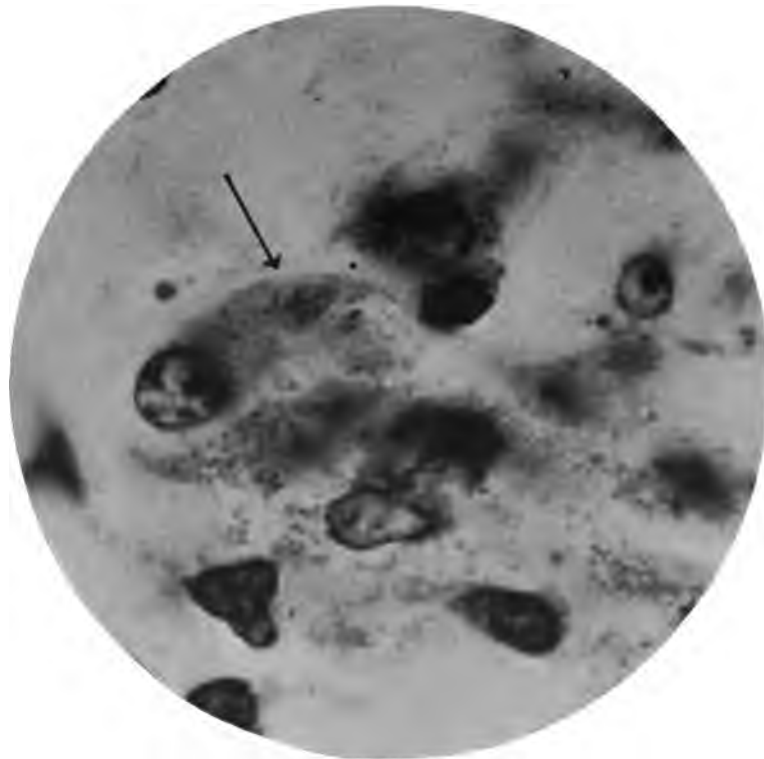
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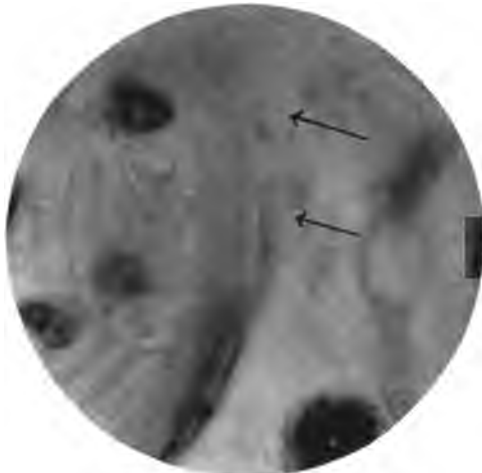
PLATE XXXI

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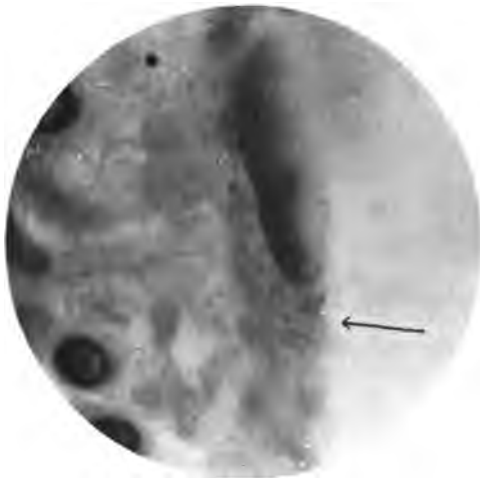
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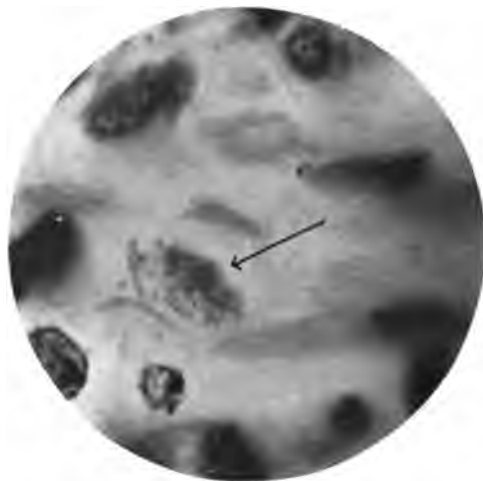
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PLATE XXXII
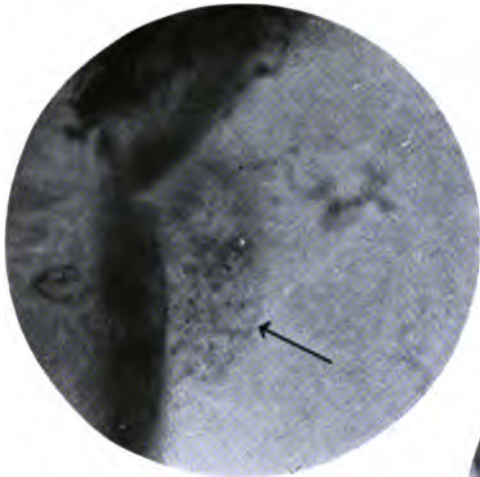
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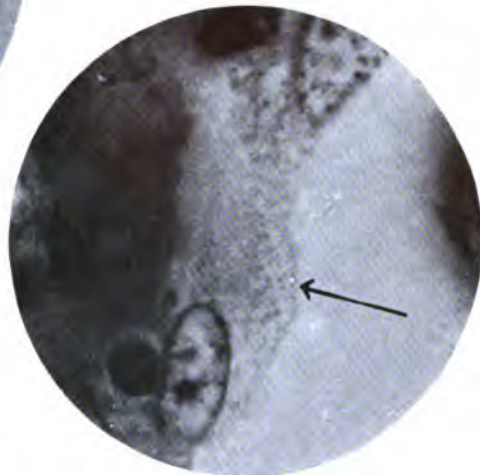
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Plate XXXIII

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Plate XXXIV